Effects of Substituting Dietary Ryegrass with Licorice Residue on Growth Performance, Meat Quality, Serum Biochemistry and Cecal Microbiota of Rabbits

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Posted Date: 26 May 2026

doi: 10.20944/preprints202605.1776.v1

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 doi:10.20944/preprints202605.1776.v1

Article

Effects of Substituting Dietary Ryegrass with Licorice Residue on Growth Performance, Meat Quality, Serum Biochemistry and Cecal Microbiota of Rabbits

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Abstract

This research aimed to determine the effects of replacing ryegrass (*Lolium perenne* L.) with licorice residue (LR) on growth performance, slaughtering performance, meat quality, serum biochemistry and cecal flora. Five pelleted rations were formulated with different LR proportions at 0%, 15%, 30%, 45%, and 60% on a dry weight basis. One hundred and twenty 35-day old Ira rabbits with nearly uniform body weight were randomly allocated into five treatment groups, each with 24 rabbits for this experiment. The adaptation (pre-feeding) phase spanned 7 days, followed by a 49-day experimental period. The results indicated that the group receiving 15% LR achieved the highest body weight by day 49 ($p < 0.05$). Over the 0-49 day interval, the average daily gain (ADG) for the 15% LR group was notably greater compared to the 0%, 45%, and 60% LR groups ($p < 0.05$), while its feed-to-gain ratio (F/G) was significantly lower than that of the 45% and 60% groups ($p < 0.05$). Additionally, the pre-slaughter live weight of the 15% LR group was substantially higher than all other groups ($p < 0.05$).The 15% LR group had a significantly lower drip loss than the 45% and 60% LR groups and a significantly higher pH value (24 h) than the 30%, 45%, and 60% LR groups ($p < 0.05$). The glutathione peroxidase (GSH-Px) in the 15% LR group was significantly lower than that in the 30% and 45% LR groups ($p < 0.05$). The superoxide dismutase (SOD) activity in the experimental groups was significantly higher than that in the control group ($p < 0.05$). The LR did not significantly affect the dominant microbial communities at the phylum and genus levels ($p > 0.05$), but it promoted the colonization of specific flora. It appears that replacing 15% of ryegrass with LR in pelleted feed was the optimal rate in making the pellet diets for rabbits to achieve a superior production performance, high-quality meat products in Ira rabbits. However, further research is needed to investigate the effect of feeding LR on growth performance, carcass traits and meat quality of other herbivorous livestock.

Keywords: licorice residue; reygrass; meat rabbit; growth performance; meat quality

1. Introduction

Licorice (Glycyrrhiza glabra L.) is a perennial herb belonging to the Fabaceae botanical family. Licorice is commonly cultivated or grows naturally in dry and semi-dry regions in the world. The plant is naturally adapted to dry, low-rainfall, and sun-rich environments through traits such as small leaves with a waxy cuticle and developed root system that can efficiently absorb water from deep soil [1]. As an very important traditional Chinese medicine, its consumption in traditional Chinese medicine prescriptions is huge [2]. It is also used as a natural sweetener agent and a diuretic in Western countries [3], while licorice extract is commonly used to treat allergic inflammation in Asia [4].

 doi:10.20944/preprints202605.1776.v1

After the industrial extraction of bioactive compounds like glycyrrhizic acid and polysaccharides from licorice, a solid waste known as Licorice Residue (LR) is generated. With the widespread application of licorice extract in industries such as pharmaceuticals and food, a large amount of LR is also generated during the licorice processing At present, the general measures for treating LR are landfilling, open-air stacking, and incineration, which can cause serious environmental pollution and resource waste in the area. Only a small amount of LR has been used to produce activated carbon or ethanol [5,6]. The LR contains large amounts of cellulose, hemicellulose, as well as small amounts of residual flavonoid medicinal components [7]. These chemical characteristics make LR a potential fiber source for herbivores. Dietary fiber is of critical importance. Cecal microorganisms hydrolyze cellulose to produce soluble sugar, and fermentation of these sugars produces volatile fatty acids and microbial protein, which are absorbed and utilized by epithelial cells in the large intestine, providing 30% - 50% of the energy of rabbits [8]. Therefore, the source and proportion of fiber are key factors affecting the nutrition of rabbits [9]. Previous studies have confirmed that licorice extract can enhance the antioxidant capacity of rabbit products during processing and storage, thereby better preserving their nutritional value and extending shelf life [10]. Additionally, it enhances both the growth performance and antioxidant capacity in chickens [11]. Given the important role of fiber in the physiological metabolism of rabbits, as well as the compositional similarity between LR and Licorice, we hypothesize that LR has potential value as an unconventional feed resource, and may positively affect the growth performance, meat quality, serum biochemical indices, and cecal microbiota of rabbits. Relevant studies have proposed that grinding it into pellet feed is a feasible utilization method [12]. Therefore, this study aimed to investigate growth performance, meat quality, serum biochemical indices and cecal microbiotain rabbits fed with supplementing pelleted diets with different proportions of LR as a substitute for ryegrass, and to determine the optimal addition ratio of LR in rabbit diets. The results of this study could provide scientific information for the feed utilization of LR.

2. Materials and Methods

2.1. Experimental Material

LR was purchased from Xinjiang Alar Xinnong Licorice Industry Co., Ltd and ryegrass (Lolium perenne L.) was harvested from the campus of Tarim University in Alar, Xinjiang (longitude 81°31′E, latitude 40°56′N). Other feed ingredients such as corn, soybean meal, and wheat bran were bought from Aksu Tiankang Livestock Co., Ltd. The chemical composition of LR and ryegrass is presented in Table 1.

Table 1. Nutrient content (% on air-dry basis).

Items	LR [1]	Regrass
DM [2]	87.53	93.2
CP [3]	7.77	8.57
NDF [4]	68.17	61.79
ADF [5]	37.07	33.90
EE [6]	3.99	2.60
Ca	0.25	0.29
TP [7]	0.14	0.16

[1] LR = licorice residue; [2] DM = dry matter; [3] CP = crude protein; [4] NDF = neutral detergent fiber; [5] ADF = acid detergent fiber; [6] EE = ether extract; [7] TP = total phosphorus.

The basal diet for rabbits was formulated according to the recommendations in *Nutrition of the Rabbit* [13]. The diets were processed into cylindrical pellets with a diameter of 6 mm. Its composition and nutritional levels are presented in Table 2.

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Table 2. Ingredient and nutrient levels of basal diets (dry basis).

Items [1]	0% LR	15% LR	30% LR	45% LR	60% LR
Ingredients, %					
LR	0.00	3.00	6.00	9.00	12.00
Ryegrass	20.00	17.00	14.00	11.00	8.00
Alfalfa meal	5.00	5.00	5.00	5.00	5.00
Corn	26.00	26.00	26.00	26.00	26.00
$CaHPO_4$	0.80	0.80	0.80	0.80	0.80
Rice bran	17.70	17.70	17.70	17.70	17.70
Bran	16.00	16.00	16.00	16.00	16.00
Soya bean meal	11.00	11.00	11.00	11.00	11.00
NaCl	0.50	0.50	0.50	0.50	0.50
Premix [2]	3.00	3.00	3.00	3.00	3.00
Total	100.00	100.00	100.00	100.00	100.00
Nutrient levels, %					
DE, (MJ/kg) [3]	9.73	9.72	9.71	9.70	9.69
CP [4]	14.75	14.73	14.70	14.68	14.66
CF [5]	10.28	10.29	10.30	10.31	10.32
NDF [6]	28.59	28.78	28.97	29.16	29.36
ADF [7]	13.78	13.87	13.97	14.06	14.16
ADL [8]	4.14	4.17	4.20	4.24	4.27
Ash [9]	5.42	5.40	5.37	5.34	5.32
EE [10]	2.77	2.81	2.86	2.90	2.94
Ca	0.88	0.86	0.84	0.82	0.81
TP [11]	0.47	0.47	0.47	0.48	0.48
Lys [12]	0.81	0.80	0.79	0.79	0.78
Met+Cys [13]	0.52	0.51	0.51	0.50	0.50

[1] The 0% LR group served as the control. The designations 15% LR, 30% LR, 45% LR, and 60% LR refer to the experimental diets in which 15, 30, 45, 60% of the ryegrass meal was substituted with LR. [2] Premix is provided per kg of the diet：vitamin A 8000 IU; vitamin D_3 1500 IU; vitamin E 50 mg; vitamin K_3 2 mg; vitamin B_2 3 mg; Fe 50 mg; Mn 10 mg; Se 0.05 mg; Cu 10 mg; Zn 50 mg. [3] DE and amino acid are calculated values, others are measured values. [4] CP = crude protein; [5] CF = Crude Fiber; [6] NDF = neutral detergent fiber; [7] ADF = acid detergent fiber; [8] ADL = acid detergent lignin; [9] Ash = crude Ash; [10] EE = ether extract; [11] TP = total phosphorus; [12] Lys = Lysine; [13] Met+Cys = Methionine+Cysteine.

The contents of crude protein (CP), crude fiber (CF), ash, ether extract (EE), neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), phosphorus (P), and amino acids were determined following the corresponding the methods 984.13, 962.09, 942.05, 920.39, 2002.04, 973.18, 968.08, 965.17, and 982.30 of the Association of Official Analytical Chemists (AOAC) [13]. Calculation of the gross energy (GE) value of the feed followed the equation provided by Song et al. [15]. Meanwhile, the digestible energy (DE) was derived based on the methodology of De Blas and Wiseman [16]. The energy values were calculated using the following formulas:

$$DE\ (MJ/kg\ DM) = (-1188 + 1.01 \times GE + 20.9 \times CP - 38.5 \times CF - 53.3 \times ADF) \times 0.0041868 \quad (1)$$

$$GE\ (MJ/kg\ DM) = [4153 + (56 \times EE) + (15 \times CP) - (44 \times Ash)] \times 0.0041868 \quad (2)$$

2.2. Experimental Design and Feeding Management

Approval for the experimental protocol was obtained from the Scientific Ethics Committee of Tarim University (approval number: PA20250620001). The experiment was carried out at Tarim University Animal Experiment Station. One hundred and twenty 35-day old Ira rabbits with nearly uniform body weight (1.00 ± 0.02 kg) were randomly allocated into five treatment groups (replacing

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0%, 15%, 30%, 45%, and 60% of ryegrass with licorice in the pelleted rations), each with 24 rabbits for this experiment. This experiment was conducted in a meat rabbit breeding plant with automated water supply and manure removal, controlled indoor temperature and lighting.

The experiment includes a 7-day pre-trial period and a 49 day formal-trial period. Feed and water were supplied ad libitum to the rabbits. The experimental animals are raised in professional meat rabbit cages (50 cm × 40 cm × 35 cm), with each rabbit raised in a single cage. By cleaning the cages and promptly removing feces, the breeding rabbit house was kept clean and hygienic throughout the entire experimental period.

2.3. Measurements and Methods

2.3.1. Growth Performance

The experimental rabbits were weighed before morning feeding on days 1, 25, and 49 of the formal experimental period. The values obtained were used as the initial body weight (IBW) and final body weight (FBW) for each phase of the experiment. These data were used to compute the average daily weight gain (ADG). Average daily feed intake (ADFI) was calculated based on the documented daily feed intake. The formulas for compute ADFI, ADG and feed-to-gain ratio (F/G) were showed as follows [17].

$$\text{ADG} = (\text{FBW} - \text{IBW}) / \text{number of experimental days} \quad (3)$$

$$\text{ADFI} = \text{Total feed intake during the experimental period} / \text{Number of experimental days} \quad (4)$$

$$\text{F/G} = \text{ADFI} / \text{ADG} \quad (5)$$

2.3.2. Slaughter Performance

At the end of the experimental period, 16 rabbits from each group were subjected to exsanguination through the jugular, then were skinned. The assessment of slaughter traits followed the methods outlined by Liu et al. [18].

- Slaughter Live Weight: The weight of the experimental rabbits after fasting for 12 h and immediately prior to the slaughter.
- Semi-eviscerated Weight: The weight measured after the forelimbs are removed at the carpal joints, the hindlimbs at the tarsal joints, the gastrointestinal tract (including its contents), the urogenital organs, and the head at the first cervical vertebra, along with the trachea and esophagus. The liver (gallbladder removed), kidneys, and perirenal fat were retained.
- Full-eviscerated Weight: The weight obtained from the semi-eviscerated carcass after further removal of the heart, liver, kidneys, and perirenal fat.
- Full-eviscerated Weight: The semi-eviscerated carcass weight minus the heart, liver, kidneys, and perirenal fat.

$$\text{Semi-eviscerated Dressing Percentage (\%)} = (\text{Semi-eviscerated Weight} / \text{Slaughter Live Weight}) \times 100\% \quad (6)$$

$$\text{Full-eviscerated Dressing Percentage (\%)} = (\text{Full-eviscerated Weight} / \text{Slaughter Live Weight}) \times 100\% \quad (7)$$

2.3.3. Meat Quality

After slaughter, samples were taken from the longissimus dorsi muscles on both sides and the biceps femoris muscles in the hind legs. Meat quality indicators listed below were determined following the protocol of Wang et al. [19].

- pH Value: A pH meter (SMART-PH818M, Dongguan Wanchuang Electronic Products Co., Ltd., China) was employed for pH determination. Measurements were taken at 45 minutes and 24

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hours post-slaughter. For each specimen, readings were collected from the top, middle, and bottom portions, and the mean value was derived.

- Meat Color: A colorimeter (Chroma Meter CR-400, Konica Minolta Sensing, Inc., Tokyo, Japan) was used to measure the redness (a*), yellowness (b*), and lightness (L*) values. For every specimen, readings were collected from the top, middle, and bottom sections, and then the mean was derived.
- Cooking Loss: The sample underwent weighing (W1), sealing in a plastic bag, and heating in a water bath at 75-80 ℃ for 1 hour. Subsequently, running water was applied to cool the sample for 30 minutes. The sample was then removed, its surface moisture was removed using filter paper, and it was weighed again (W2).

$$\text{Cooking Loss (\%)} = [(W1 - W2) / W1] \times 100\% \quad (8)$$

- Drip Loss: The external membrane and adhering fat were removed from the sample, and a portion weighing approximately 20 g was cut into a cube. A thread was used to suspend the sample, which was then enclosed in a plastic bag (with the bag opening tied tightly and enough space left inside to collect exudate) and hung at 4 ℃ for 24 hours. Subsequently, the specimen was taken out of the bag, any surface liquid was absorbed using filter paper, and it was weighed again to determine the drip loss.

$$\text{Drip Loss (\%)} = (\text{Initial sample weight} - \text{Final sample weight after hanging}) / \text{Initial sample weight} \times 100\% \quad (9)$$

- Shear Force: After being placed in a plastic pouch, the specimen was immersed in a 75-80°C water bath for 2 hours, then chilled under running tap water for 30 minutes. A block measuring 1.5 cm × 1.0 cm × 0.5 cm was cut along the direction of the muscle fibers. A meat tenderness meter (Model C-LM3A, Bulade Technology Development Co., Ltd., Beijing, China) was used to determine the shear force.

2.3.4. Serum Biochemical Parameters

Two individuals from each replicate were chosen to collect approximately 5 mL of blood per rabbit via heart puncture using the method described by Baláži et al. [20]. Blood samples were left undisturbed at ambient temperature for 30 min, then centrifuged at 1,250 × g for 10 min to isolate the serum. the serum was transfered into labeled 1.5-mL cryogenic vials and stored at -80 ℃ for subsequent analysis. A biochemical analyzer (Beckman Coulter, Inc., Brea, CA, USA) was used to measure total protein (TP), albumin (ALB), globulin (GLB), total cholesterol (TC), triglycerides (TG), blood urea nitrogen (BUN), glucose (GLU) concentrations, as well as alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities.

2.3.5. Serum Antioxidant Parameters

Serum antioxidant parameters were assessed by using commercial assay kits (Jiangsu Aidisheng Biotechnology Co., Ltd., Nanjing, China). Total antioxidant capacity (T-AOC) via the FRAP method (Cat. No. ADS-W-KY005-48); superoxide dismutase (SOD) activity via the WST-8 method (Catalog numbers: ADS-W-KY011-48); malondialdehyde (MDA) content via the thiobarbituric acid (TBA) colorimetric method (Catalog numbers: ADS-W-YH002-48); and glutathione peroxidase (GSH-Px) activity via the organic peroxide substrate method (Catalog numbers: ADS-W-G003-48). Each assay was carried out strictly following the manufacturer's protocols.

2.3.6. Determination of Cecal Microbiota

The cecum was isolated after sacrifice of the experimental rabbits. Cecal contents from the proximal, middle, and distal regions were collected, mixed and frozen without delay in dry ice and stored at −80°C separately. The samples were then shipped to Shanghai Personal Biotechnology Co., Ltd. on dry ice for DNA extraction. The microbiota sequencing was then performed using the Illumina HiSeq platform. PCR amplification of target fragments was performed, and sequencing

 doi:10.20944/preprints202605.1776.v1

libraries were constructed with the TruSeq Nano DNA LT Library Prep Kit (Illumina), after which high-throughput sequencing was carried out to evaluate microbial diversity. Utilizing the Personal Gene Cloud platform (www.genecloud.cn), the association between the cecal microbiota of rabbits and the substitution level of ryegrass meal with LR was systematically analyzed.

2.4. Statistical Analysis

Experimental data were organized with Excel 2019, while SPSS 27.0 software served for statistical analysis. One-way ANOVA and subsequent Duncan's multiple comparisons (significance set at $p < 0.05$) were conducted. Data are presented as the mean and standard error of the mean (SEM). Linear and quadratic regression analyses were employed to assess the relationship between the different dietary levels of LR and the various measured indicators in the rabbits to test significance at $p < 0.05$.

3. Results

3.1. Growth Performance

As shown in Table 3, the body weight of rabbits on 49th days in the 15% LR group was significantly higher than that in the 0%, 45%, and 60% LR groups ($p < 0.05$). During the 0-25 days period, the ADG in the 15% LR group was significantly higher than that in the 45% and 60% LR groups ($p < 0.05$), and the F/G was significantly lower than that in the 45% and 60% LR groups ($p < 0.05$). Increasing the proportion of LR resulted in significant linear trends for ADG and F/G ($p < 0.05$). During the 26-49 days period, no statistically significant differences were detected in ADFI, ADG, or F/G among the groups ($p > 0.05$). Throughout the entire 0-49 days period, no statistically significant difference in ADG was found between the 15% and 30% LR groups ($p > 0.05$), but the ADG in these two groups was significantly higher than that in the other three groups ($p < 0.05$). The F/G in the 15% LR group was not statistically significantly different from that in the 0% and 30% LR groups ($p > 0.05$) but it was significantly lower than that in the other two groups ($P < 0.05$). As the proportion of LR increased, ADFI and ADG exhibited significant quadratic trends ($p < 0.05$), while F/G showed a significant linear increasing trend ($p < 0.05$).

Table 3. Effects of replacing ryegrass with LR on growth performance of rabbits.

Item	0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*p*-value [2]		
							Treatment	Linear	Quadratic
0 d weight, g	1022.50	997.86	1027.86	1070.00	1027.86	10.972	0.357	0.290	0.803
25 d weight, g	1866.36^{b}	2083.57^{a}	1937.86^{a}	1790.00^{a}	1837.86^{a}	29.884	0.010	0.061	0.120
49 d weight, g	2544.55^{bc}	2837.86^{a}	2695.00^{ab}	2440.71^{c}	2512.86^{c}	34.963	<0.001	0.020	0.019
days 0 to 25									
ADFI [3], g/d	127.66	136.01	135.22	130.05	129.76	1.149	0.075	0.816	0.021
ADG [4], g/d	33.75^{abc}	40.46^{a}	36.40^{ab}	28.80^{c}	30.97^{bc}	1.212	0.013	0.028	0.163
F/G [5]	3.88^{ab}	3.38^{b}	3.82^{ab}	4.63^{a}	4.49^{a}	0.152	0.044	0.017	0.348
days 26 to 49									
ADFI, g/d	134.50	140.05	140.42	139.69	137.61	1.222	0.538	0.509	0.124
ADG, g/d	27.13	30.17	30.29	27.11	27.00	0.528	0.062	0.343	0.025
F/G	4.99	4.69	4.67	5.24	5.10	0.092	0.195	0.232	0.228
days 0 to 49									
ADFI, g/d	131.08^{b}	138.03^{a}	137.82^{a}	134.87^{ab}	133.68^{b}	0.702	0.003	0.620	<0.001
ADG, g/d	30.44^{bc}	35.31^{a}	33.34^{ab}	27.96^{c}	28.99^{c}	0.716	0.002	0.017	0.028
F/G	4.37^{abc}	3.93^{c}	4.17^{bc}	4.86^{a}	4.69^{ab}	0.099	0.012	0.014	0.176

[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$). [3] ADFI = average daily feed intake; [4] ADG = average daily gain; [5] F/G = feed to gain ratio.

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3.2. Slaughter Performance

According to Table 4, the 15% LR group exhibited a markedly higher slaughter live weight than the remaining groups ($p < 0.05$). All groups exhibited no significant differences in semi-eviscerated weight and full-eviscerated dressing percentage ($p > 0.05$). The semi-eviscerated dressing percentage of the 15% LR group was significantly greater than that of the 30% and 60% groups ($p < 0.05$). With increasing LR inclusion, slaughter live weight followed a significant quadratic pattern, rising initially and then declining ($p < 0.05$); meanwhile, semi-eviscerated dressing percentage showed a significant linear decreasing trend ($p < 0.05$).

Table 4. Effects of replacing ryegrass with LR on meat rabbit slaughter performance.

Item	0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*p*-value [2]		
							Treatment	Linear	Quadratic
Slaughter live weight, g	2546.67^{c}	2848.33^{a}	2680.00^{b}	2454.00^{d}	2533.33^{c}	37.767	<0.001	<0.001	<0.001
Semi-eviscerated weight, g	1321.91	1434.42	1351.52	1333.33	1256.29	22.546	0.150	0.124	0.084
Full-eviscerated weight, g	1209.68	1342.27	1247.55	1239.21	1181.99	21.960	0.182	0.278	0.103
Semi-eviscerated dressing percentage, %	51.93^{ab}	52.19^{a}	50.15^{bc}	50.89^{abc}	49.59^{c}	0.345	0.041	0.008	0.887
Full-eviscerated dressing percentage, %	47.53	48.81	46.29	47.30	46.65	0.328	0.107	0.123	0.891

[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$).

3.3. Meat Quality

Table 5 presents the results meat qulity of rabbits. For cooking loss in the longissimus dorsi, no significant difference was detected among the 15%, 30%, and 60% LR groups ($p > 0.05$), however, their values were significantly elevated relative to those of the 0% group ($p < 0.05$). Cooking loss in the leg muscle did not vary significantly across the 0%, 15%, 30%, and 45% groups ($p > 0.05$). For drip loss in the longissimus dorsi, the 15% group showed no significant difference from the 0% and 30% groups ($p > 0.05$), but its value was significantly lower than that of the other two ($p < 0.05$). Across all groups, no significant differences were found for drip loss in the leg, shear force, leg pH at 45 min and 24 h post-mortem, longissimus dorsi pH at 45 min, or longissimus dorsi b* (yellowness) ($p > 0.05$). For longissimus dorsi pH at 24 h post-mortem, the 15% and 0% groups did not differ significantly ($p > 0.05$), but the 15% group exhibited a significantly higher value than the other three groups ($p < 0.05$). The biceps femoris L* value was significantly higher in the 60% group than in the remaining four groups ($p < 0.05$). No significant differences were observed among the 15%, 30%, and 45% groups for longissimus dorsi a* (redness), leg a*, or longissimus dorsi L* ($p > 0.05$). As the dietary proportion of LR increased, longissimus dorsi pH at 24 h post-mortem and leg a* followed significant quadratic trends ($p < 0.05$), whereas the other indices displayed significant linear trends ($p < 0.05$).

Table 5. The effect of replacing ryegrass with LR on the meat quality of rabbits.

Item		0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*p*-value [2]		
								Treatment	Linear	Quadratic
Drip loss, %	Back	23.36^{c}	26.69^{b}	28.91^{ab}	30.21^{a}	29.72^{ab}	0.583	<0.001	0.002	0.553
	Leg	34.44^{c}	36.16^{bc}	35.43^{bc}	38.45^{ab}	39.61^{a}	0.550	0.011	0.003	0.992
	Back	8.54^{bc}	7.33^{c}	11.27^{abc}	12.11^{ab}	13.44^{a}	0.701	0.024	0.010	0.151

 doi:10.20944/preprints202605.1776.v1

Cooking loss, %	Leg	8.64	8.16	7.34	8.51	9.54	0.364	0.443	0.410	0.108
Shear	Back	15.53^{b}	20.93^{ab}	25.55^{a}	14.65^{b}	28.09^{a}	1.477	0.006	0.046	0.958
force, N	Leg	34.16	31.14	32.42	33.88	28.29	1.172	0.518	0.287	0.618
pH	Back	6.11	6.17	6.16	6.05	5.96	0.031	0.072	0.024	0.073
(45min)	Leg	5.96	5.90	5.97	5.89	5.97	0.027	0.522	0.863	0.412
pH	Back	5.73^{a}	5.59^{a}	5.13^{b}	5.21^{b}	5.19^{b}	0.043	<0.001	<0.001	<0.001
(24h)	Leg	5.28	5.28	5.28	5.28	5.28	0.032	0.246	0.164	0.247
Meat	L*	37.56^{ab}	35.44^{c}	36.00^{c}	36.22^{bc}	37.78^{a}	0.255	0.004	0.005	0.113
color	a*	3.78^{bc}	4.56^{a}	4.44^{a}	4.33^{ab}	3.67^{c}	0.101	0.006	<0.001	0.765
(Back)	b*	6.78	6.00	7.33	6.44	7.33	0.184	0.083	0.210	0.447
Meat	L*	30.22^{b}	29.44^{b}	29.67^{b}	29.67^{b}	31.89^{a}	0.237	0.001	0.008	0.340
color	a*	4.67^{b}	5.56^{a}	5.44^{a}	5.11^{ab}	4.67^{b}	0.112	0.013	0.525	0.001
(Leg)	b*	5.67^{a}	4.33^{b}	6.33^{a}	5.56^{a}	6.33^{a}	0.205	0.004	0.040	0.317

[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$).

3.4. Serum Biochemical Parameters

As presented in Table 6, the ALP activity in the 15% LR group showed no statistically significant difference compared to the 30% and 45% LR groups ($p > 0.05$), but it was significantly lower than in the 0% LR group ($p < 0.05$). The BUN concentration in the 15% LR group was not statistically significantly different from that in the 0% and 60% LR groups ($p > 0.05$), but it was significantly lower than that in the 30% and 45% LR groups ($p < 0.05$). The other measured parameters showed no statistically significant differences among the groups ($p > 0.05$). As the proportion of liquorice residue increased, ALP concentration exhibited an initial decrease followed by a subsequent increase, whereas BUN concentration showed the opposite pattern (an initial increase followed by a decrease). Both parameters displayed a significant quadratic trend ($p < 0.05$).

Table 6. The effect of replacing ryegrass with LR on the biochemical indicators of meat rabbit serum.

Item	0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*p*-value [2]		
							Treatment	Linear	Quadratic
GLU [3], mmol/L	7.73	9.87	9.03	8.67	10.40	0.422	0.325	0.178	0.923
ALT [4], U/L	49.77	50.83	50.80	50.57	48.93	1.852	0.884	0.701	0.357
ALP [5], U/L	147.33^{a}	97.33^{bc}	80.67^{c}	104.00^{bc}	124.67^{ab}	7.218	0.007	0.246	<0.001
ALB [6], g/L	39.47	38.63	39.70	40.70	41.03	0.393	0.324	0.079	0.487
BUN [7], mmol/L	5.27^{bc}	5.20^{c}	5.97^{b}	7.73^{a}	5.50^{bc}	0.264	<0.001	0.001	0.002
TP [8], g/L	53.20	49.17	51.13	53.53	55.87	0.828	0.077	0.066	0.040
TC [9], mmol/L	1.53	1.30	1.13	1.37	1.60	0.077	0.343	0.710	0.057
TG [10], mmol/L	1.23	1.03	0.88	0.74	1.35	0.110	0.452	0.950	0.110
A/G [11]	2.91	3.70	3.49	3.21	2.79	0.121	0.058	0.292	0.010

[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$). [3] GLU = glucose; [4] ALT = alanine aminotransferase; [5] ALP = alkaline phosphatase; [6] ALB = albumin; [7] BUN = blood urea nitrogen; [8] TP = total protein; [9] TC = total cholesterol; [10] TG = triglyceride; [11] A/G = albumin/globulin ratio.

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3.5. Serum Antioxidant Parameters

As presented in Table 7, the MDA content in the 15% LR group showed no statistically significant difference compared to the 0%, 30%, and 45% LR groups ($p > 0.05$), but it was significantly lower than that in the 60% LR group ($p < 0.05$). The SOD activity was significantly higher in all licorice-residue treatments than that in the control group ($p < 0.05$). For GSH-Px activity, the value in the 15% LR group was not statistically significantly different from that in the 0% and 60% groups ($p > 0.05$), but it was markedly lower than those in the 30% and 45% groups ($p < 0.05$). Regarding T-AOC level, no significant differences were detected between the 15% group and the other four groups ($p > 0.05$). As the proportion of LR increased, the MDA content, SOD activity, and GSH-Px activity exhibited significant linear upward trend ($p < 0.05$), while the T-AOC level showed a significant quadratic trend of first rising and then falling ($p < 0.05$).

Table 7. The effect of replacing ryegrass with LR on the antioxidant indicators of rabbit serum.

Item	0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*p*-value [2]		
							Treatment	Linear	Quadratic
MDA [3], nmol/mL	2.65^{c}	3.03^{bc}	2.97^{bc}	3.08^{b}	3.86^{a}	0.116	<0.001	<0.001	0.064
SOD [4], U/mL	159.78^{c}	223.23^{ab}	213.66^{b}	242.39^{ab}	262.04^{a}	10.669	0.005	<0.001	0.376
GSH-Px [5], U/mL	157.78^{c}	157.14^{c}	182.05^{a}	181.75^{a}	178.92^{bc}	4.335	0.034	0.014	0.154
T-AOC [6], U/mL	2.26^{b}	2.54^{ab}	2.83^{a}	2.92^{a}	2.66^{a}	0.076	0.017	0.009	0.014

[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$). [3] MDA = malondialdehyde; [4] SOD = superoxide dismutase; [5] GSH-Px = glutathione peroxidase; [6] T-AOC = total antioxidant capacity.

3.6. Cecal Microbiota

3.6.1. Venn Diagram

Figure 1 illustrates the similarities and differences in cecal microbiota among the different groups of rabbits. The number of unique Amplicon Sequence Variants (ASVs) were 5,609 for the 0% LR group, 6,063 for the 15% LR group, 6,514 for the 30% LR group, 7,060 for the 45% LR group, and 6,821 for the 60% LR group.

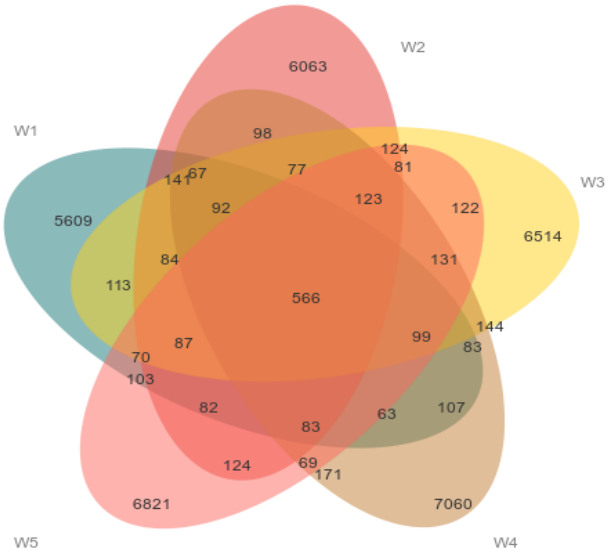

Figure 1. Venn Diagram of ASVs Distribution. W1 to W5 represent the 0%, 15%, 30%, 45%, and 60% LR groups, respectively.

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3.6.2. LEfSe Analysis

Based on Figure 2, the LEfSe analysis revealed that no statistically significantly discriminative microbial taxa were identified in the 15%, 30%, and 60% LR groups ($p > 0.05$). In contrast, a single discriminative taxon was detected in the 0% LR group ($p < 0.05$), while seven discriminative taxa were identified in the 45% LR group ($p < 0.05$).

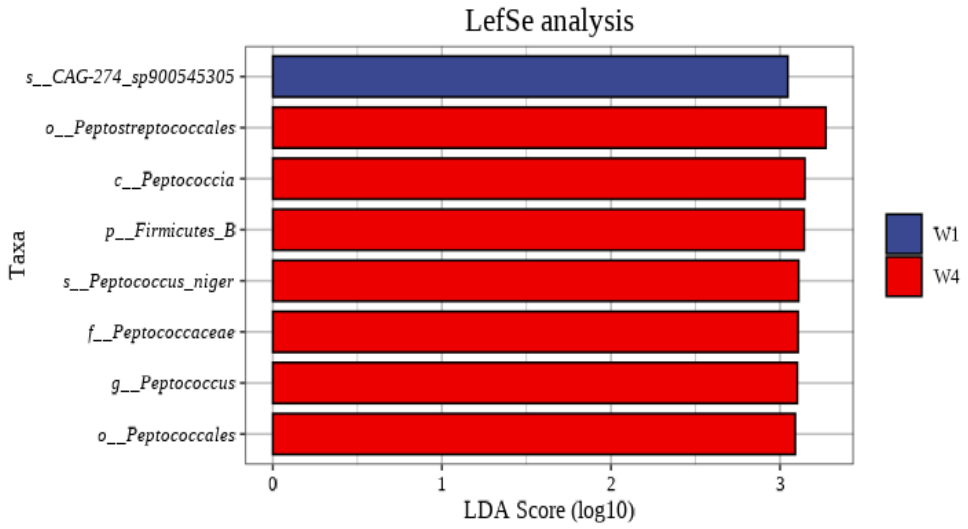

Figure 2. Linear discriminant analysis effect size (LEfSe) analysis of differentially abundant taxa between W1 and W4 groups.

3.6.3. Relative Abundance of Cecal Microbiota at the Phylum Level (Top 10)

As shown in Figure 3 and Table 8, Firmicutes_A, Bacteroidota, and Verrucomicrobiota were the dominant phyla. The combined relative abundances of these three phyla in the 0%, 15%, 30%, 45%, and 60% LR groups were 83.50%, 77.56%, 85.77%, 84.32%, and 85.82%, respectively. In the cecal microbiota of rabbits, the relative abundance of Cyanobacteria was significantly changed by substituting ryegrass with LR. Specifically, the 60% LR group showed a significantly higher relative abundance of Cyanobacteria compared to all other groups ($p < 0.05$).

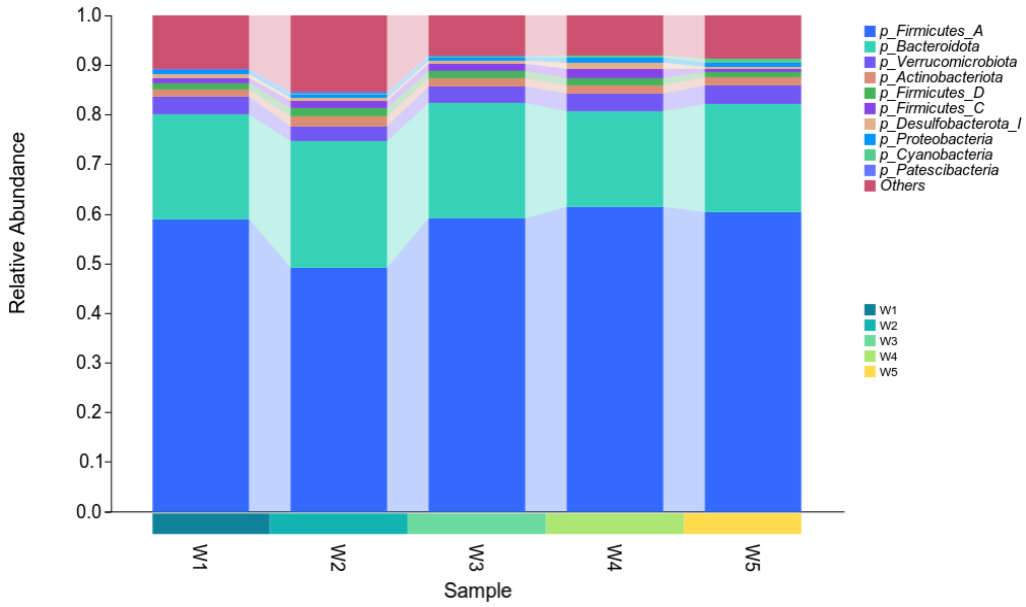

Figure 3. Relative Abundance of Cecal Microbiota at the Phylum Level(Top 10) W1 to W5 represent the 0%, 15%, 30%, 45%, and 60% LR groups, respectively.

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Table 8. The effect of replacing ryegrass with LR on the relative abundance of bacterial phyla in the cecum of rabbits (%).

Item	0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*p*-value [2]		
							Treatment	Linear	Quadratic
Firmicutes_A	58.78	49.22	59.04	61.29	60.31	1.588	0.079	0.126	0.391
Bacteroidota	21.34	25.36	23.33	19.44	21.82	1.617	0.870	0.707	0.740
Verrucomicrobiota	3.38	2.98	3.40	3.59	3.69	0.605	0.998	0.813	0.899
Actinobacteriota	1.60	2.01	1.66	1.46	1.62	0.153	0.885	0.690	0.818
Firmicutes_D	1.13	1.71	1.41	1.65	1.22	0.111	0.408	0.870	0.134
Firmicutes_C	1.21	1.50	1.39	1.70	0.46	0.169	0.155	0.242	0.057
Desulfobacterota_I	0.80	0.55	0.55	1.37	0.58	0.138	0.284	0.686	0.818
Proteobacteria	0.65	0.71	0.73	0.89	0.72	0.048	0.655	0.389	0.472
Cyanobacteria	0.12[b]	0.17[b]	0.12[b]	0.40[b]	0.76[a]	0.075	0.003	<0.001	0.025
Patescibacteria	0.12	0.47	0.31	0.20	0.13	0.055	0.212	0.487	0.085

[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$).

3.6.4. Relative Abundance of Cecal Microbiota at the Genus Level (Top 20)

As shown in Figure 4 and Table 9, Faecousia, SFMI01, and CAG-485 were identified as the dominant genera. The combined relative abundances of these three genera in the 0%, 15%, 30%, 45%, and 60% LR groups were 16.89%, 11.93%, 21.01%, 12.17%, and 16.77%, respectively. Replacing ryegrass with LR significantly influenced the cecal microbial abundance of the genus Phascolarctobacterium_A. More precisely, the relative abundances in the 0%, 30%, and 45% LR groups were markedly greater than those in the 15% and 60% groups ($p < 0.05$).

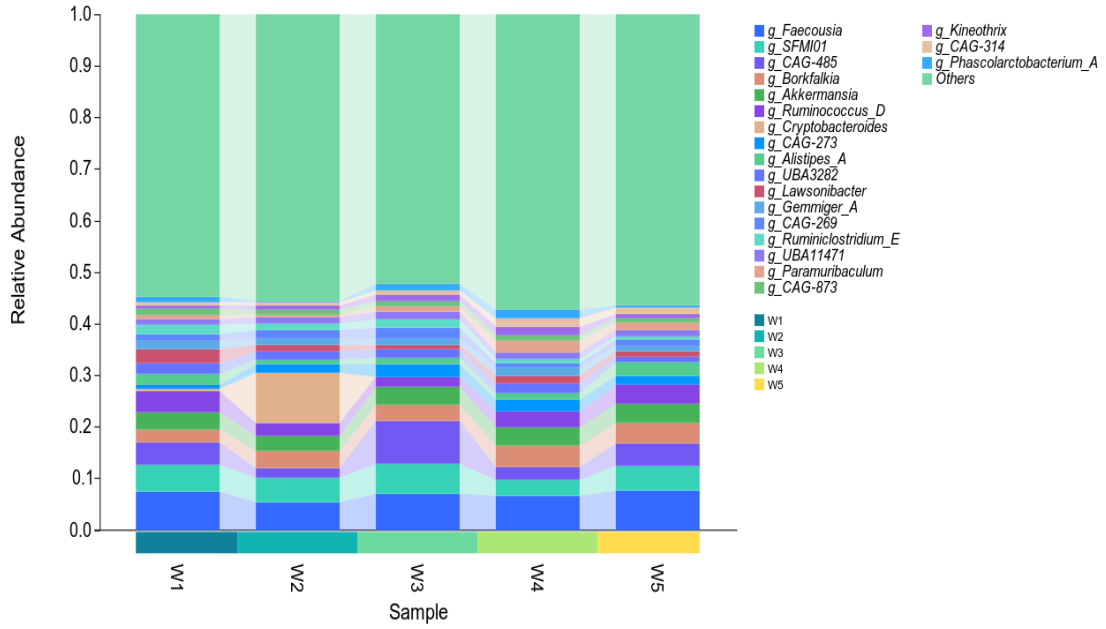

Figure 4. Relative Abundance of Cecal Microbiota at the Genus Level(Top 20). W1 to W5 represent the 0%, 15%, 30%, 45%, and 60% LR groups, respectively.

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Table 9. The effect of replacing ryegrass with LR on the relative abundance of bacterial genera in the cecum of rabbits (%).

Item	0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*P*-value [2]		
							Treatment	Linear	Quadratic
Faecousia	7.47	5.35	7.04	6.58	7.65	0.472	0.611	0.656	0.328
SFMI01	5.15	4.74	5.84	3.16	4.75	0.487	0.575	0.523	0.958
CAG-485	4.27	1.84	8.13	2.43	4.36	0.920	0.222	0.899	0.652
Borkfalkia	2.54	3.37	3.25	4.09	3.89	0.359	0.741	0.240	0.744
Akkermansia	3.37	2.97	3.39	3.55	3.68	0.603	0.998	0.817	0.896
Ruminococcus_D	4.08	2.40	1.96	3.16	3.85	0.511	0.701	0.941	0.194
Cryptobacteroides	0.34	9.78	0.00	0.01	0.00	1.391	0.066	0.208	0.345
CAG-273	0.99	1.58	2.42	2.23	1.57	0.231	0.317	0.272	0.085
Alistipes_A	2.02	0.96	1.24	1.39	2.80	0.261	0.158	0.248	0.030
UBA3282	2.07	1.54	1.69	1.84	0.94	0.257	0.761	0.346	0.756
Lawsonibacter	2.72	1.20	0.82	1.48	1.01	0.275	0.194	0.101	0.160
Gemmiger_A	1.73	1.39	1.41	1.46	1.08	0.205	0.935	0.472	0.971
CAG-269	1.09	1.56	2.04	0.86	1.23	0.192	0.367	0.763	0.262
Ruminiclostridium_E	1.99	1.22	1.60	0.98	0.73	0.213	0.390	0.092	0.988
UBA11471	1.01	1.42	1.37	1.15	1.14	0.149	0.929	0.998	0.490
Paramuribaculum	0.80	0.33	1.04	2.27	1.60	0.247	0.089	0.033	0.946
CAG-873	1.21	1.09	1.15	1.15	0.74	0.194	0.961	0.588	0.737
Kineothrix	0.77	0.70	1.25	1.54	0.81	0.146	0.305	0.369	0.204
CAG-314	0.46	0.61	0.68	1.80	1.26	0.222	0.298	0.089	0.854
Phascolarctobacterium_A	1.14^{a}	0.22^{b}	1.28^{a}	1.53^{a}	0.43^{b}	0.154	0.003	0.851	0.134

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[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$).

3.6.5. Alpha Diversity Analysis

As shown in Figure 5 and Table 10, regarding species richness, the median values and overall distributions of the Chao1 and Observed_species indices were highest in the 45% LR group and lowest in the 15% LR group, while the 0%, 30%, and 60% LR groups exhibited intermediate levels. For sequencing coverage, the median Good's coverage index was highest in the 0% LR group and lowest in the 30% LR group, with the 15%, 45%, and 60% LR groups showing relatively concentrated distributions. Concerning comprehensive diversity, the median values of the Simpson, Shannon, and Pielou_e indices were higher in the 45% and 60% LR groups and lower in the 15% LR group. As the proportion of LR increased, the species richness indices of the cecal microbiota, Chao1 and Observed_species, showed a significant linear increasing trend ($p < 0.05$), while no statistically significant effects were observed on the other Alpha diversity indices ($p > 0.05$).

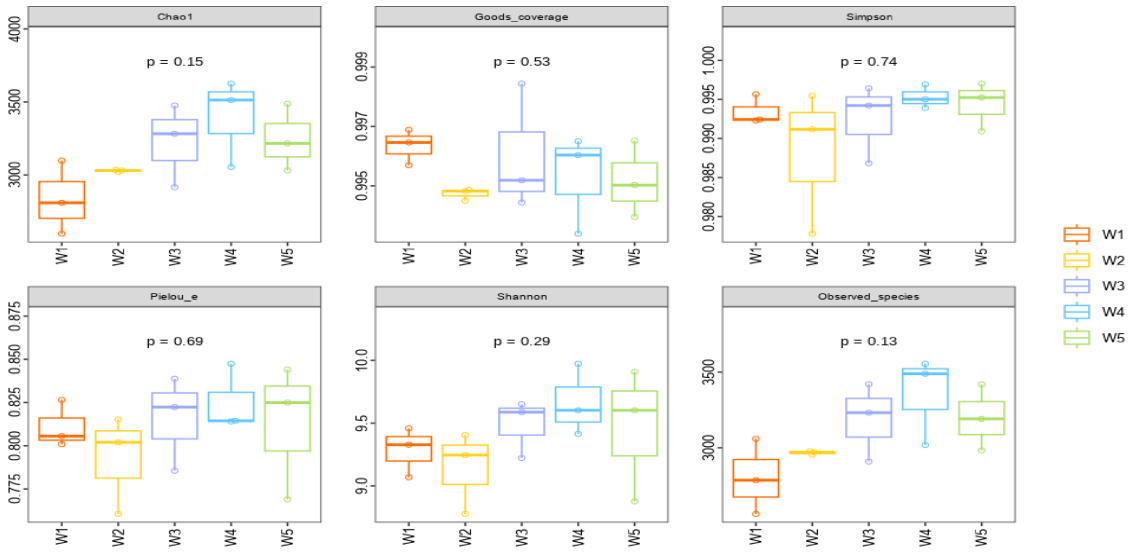

Figure 5. Boxplots of Alpha Diversity Indices. W1 to W5 represent the 0%, 15%, 30%, 45%, and 60% LR groups, respectively. The same nomenclature applies to the figures below.

Table 10. Alpha diversity index analysis.

Item	0% LR	15% LR	30% LR	45% LR	60% LR	SEM [1]	*P*-value [2]		
							Treatment	Linear	Quadratic
Chao1	2835.51	3029.16	3225.05	3397.87	3245.37	73.850	0.113	0.022	0.198
Coverage	0.9963	0.9947	0.9960	0.9953	0.9952	0.0003	0.616	0.494	0.757
Simpson	0.9935	0.9881	0.9925	0.9953	0.9944	0.001	0.488	0.349	0.515
Pielou_e	0.8110	0.7926	0.8156	0.8253	0.8127	0.007	0.681	0.480	0.978
Shannon	9.29	9.14	9.49	9.66	9.46	0.087	0.421	0.183	0.703
Observed_species	2802.67	2967.00	3187.00	3354.00	3197.67	71.948	0.093	0.018	0.191

[1] SEM = standard error of the mean; [2] Mean values with different superscripts in the same row were significantly different ($p < 0.05$).

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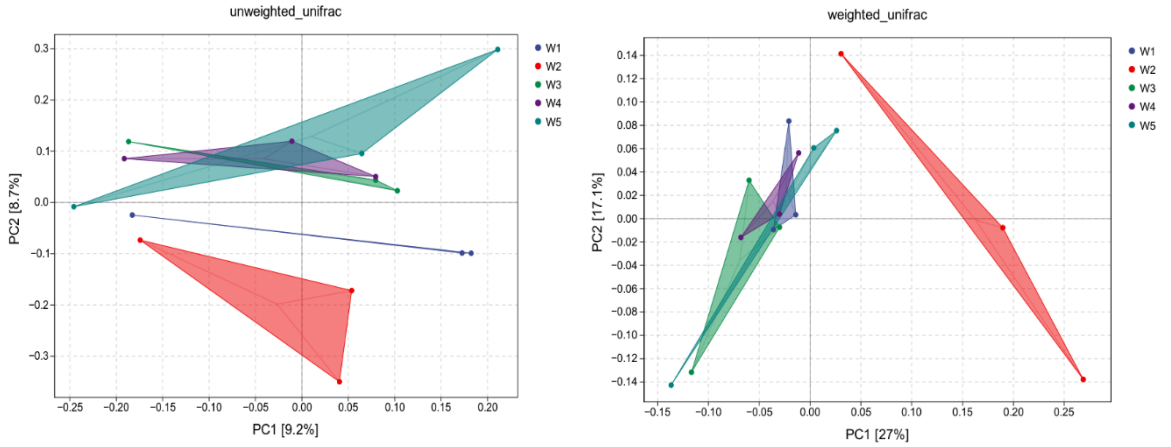
3.6.6. Beta Diversity Analysis

As shown in Figures 6 (a, b), Principal Coordinates Analysis (PCoA) based on unweighted and weighted UniFrac distances was performed. In the unweighted analysis, principal components PC1 and PC2 accounted for 9.2% and 8.7% of the sample variation; in the weighted analysis, the corresponding values were 27% and 17.1%. Samples from the 15% LR group clustered distinctly from those of the other groups. This indicates that, regardless of whether species abundance was considered (weighted vs. unweighted), the microbial community structure of the 15% LR group was distinctly different from the others, identifying it as the "core divergent group."



(a) **(b)**

Figure 6. Beta Diversity Analysis. W1 to W5 represent the 0%, 15%, 30%, 45%, and 60% LR groups, respectively. (**a**) Unweighted UniFrac PCoA plot, where PC1 and PC2 explain 9.2% and 8.7% of the total variation, respectively; (**b**) Weighted UniFrac PCoA plot, where PC1 and PC2 explain 27% and 17.1% of the total variation, respectively.

3. Discussion

According to the findings of this trial, the weight and the ADG during the 0-25 day period were highest in the 15% LR group. This may be attributed to trace amounts of flavonoid compounds in LR, such as isoliquiritigenin, which can promote gastrointestinal motility and thereby improve growth [21]. The greater ADFI and ADG observed in the 15% LR group during the 0-49 day period compared to other groups may be due to the natural sweetness of LR acting as a feeding attractant to increase palatability [22,23]. This may also be related to the immunomodulatory effects of bioactive components such as licorice polysaccharides and flavonoids, enhancing the immunity of the rabbits. This finding aligns with the similar effects on growth performance observed for curcumin in rabbits [24]. The declining trend in various indicators with increasing LR proportion is likely due to the elevated fiber content, which reduces diet palatability [25]. The slaughter performance indicators, including semi-eviscerated weight, full-eviscerated weight, and full-eviscerated dressing percentage, corresponded with the growth performance data, exhibiting a quadratic trend, with the 15% LR group showing better outcomes. This is closely related to their superior growth status. This trend in carcass indicators is similar to that reported in previous research where licorice cake was added to the diet of lambs [26]. Cooking loss is an important parameter for evaluating rabbit meat quality. During the cooking process, meat proteins denature, causing muscle fiber contraction and a reduced ability of the denatured proteins to bind water, leading to the expulsion of water from the meat structure [27]. The linear increasing trend in cooking loss for both leg and longissimus dorsi muscles with increasing LR proportion might be attributed to excessive LR impairing digestion and absorption, inhibiting muscle protein synthesis, resulting in a less dense gel network and reduced water-holding capacity. The lower drip loss in the 15% LR group compared to the control, with values increasing again at higher substitution levels, follows a trend similar to that reported in a trial using

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mulberry leaf powder in rabbit diets [28]. This could be due to excessive fiber components disrupting the microscopic structure of the meat.

Post-slaughter, pH at 24 h is typically lower than that at 45 min due to glycogen breakdown and lactic acid production [29]. No significant differences were observed in pH values at the same time point and within the same muscles among groups in this study, the overall pattern is consistent with findings in rabbits fed olive cake [30]. The L* and a* values for both longissimus dorsi and leg muscles showed consistent trends. The initial decrease followed by an increase in L* value might be explained by flavonoid compounds in LR inhibiting myoglobin oxidation, thereby stabilizing the L* value [10]. Simultaneously, they may reduce the conversion of myoglobin to metmyoglobin, helping to maintain the bright red color (higher a*). However, the subsequent increase in fiber content at higher substitution levels might induce digestive stress, accelerating muscle oxidation, which could explain the observed trend in a* value [31].

Serum biochemical parameters reflect the health status of the organism. In this study, the levels of ALP and BUN exhibited quadratic trends in response to increasing dietary LR. Research has found that licorice extract can reduce ALP activity [32]. This suggests that the active components, such as glycyrrhizic acid, present at lower substitution levels may exert a certain hepatoprotective effect. With stable hepatocyte function, the synthesis and release of ALP remain at normal levels. Conversely, when the proportion of LR is excessively high, the metabolic burden on the liver increases, potentially leading to hepatocyte damage and increased membrane permeability, thereby causing more ALP to be released into the bloodstream and elevating its activity [33]. Serum urea concentration is primarily associated with protein metabolism and renal function. An appropriate amount of LR may regulate nitrogen metabolism in rabbits, improving the utilization efficiency of dietary protein within a certain range. This allows more nitrogen to be directed towards the synthesis of body proteins, resulting in decreased serum urea concentration. The observed increase in urea concentration at higher substitution levels could be due to excessive metabolic stress on the liver and kidneys induced by LR, potentially impairing the timely conversion of ammonia to urea for excretion [34]. The T-AOC and SOD activity are important indicators for assessing the antioxidant capacity of rabbits [35]. Furthermore, SOD and GSH-Px play crucial roles in cellular defense against reactive oxygen species and other oxidants [36]. The MDA content showed a linear increase with the rising proportion of LR in this experiment. This could be attributed to the altered fiber composition inducing oxidative stress damage in the rabbits [37]. Compared with the control group, all treatment groups showed higher SOD activity and T-AOC level. The activities of GSH-Px and SOD showed linear increasing trends with the increasing LR proportion. This is likely due to the residual flavonoid compounds in LR enhancing the body's antioxidant capacity [38], a result that aligns with the findings of Guo et al. [39], where feeding 4.5% licorice root extract improved the antioxidant status of meat sheep.

Approximately 40% of the total digestive tract volume in rabbits is made up by the cecum, which also hosts a highly active microbial community essential for nutrient digestion and absorption [40,41]. The variation in the number of unique ASVs reflected the regulatory effect of LR on the cecal microbiota. The 45% LR group showed the greatest number of unique ASVs, which might be attributed to the bioactive components in LR altering the intestinal microenvironment and promoting the colonization of specific bacterial groups. The decrease in unique ASVs in the 60% LR group could be owing to the high content of LR in the diet, which increases the intestinal metabolic burden and inhibits the proliferation of some bacterial taxa [42]. The LEfSe analysis further demonstrated that the 45% LR group contained seven significantly discriminative taxonomic units, primarily belonging to orders such as Peptostreptococcales and families like Peptococcaceae. Fiber degradation and short-chain fatty acid production are commonly linked to these bacterial groups.

Studies have found that the phylum-level structure of the cecal microbiota in rabbits remains stable, predominantly composed of Firmicutes, Bacteroidota, and Verrucomicrobiota [43]. The combined relative abundance of these phyla approached 80% in all groups, consistent with other research findings [44]. Bacteroidota help maintain gut microbial homeostasis and prevent diarrhea

 doi:10.20944/preprints202605.1776.v1

by competitively inhibiting pathogen proliferation [45]. The cellulose-degrading bacteria within the Firmicutes phylum, such as Ruminococcus albus and Ruminococcus flavefaciens, are key microbial groups for cecal fiber digestion; changes in their abundance can affect the digestive utilization of fibrous feed by rabbits [46]. Verrucomicrobiota, which decompose water-soluble carbohydrates, are closely related to gut health [47].

The genus-level structure of the cecal microbiota in rabbits was relatively stable, with Faecousia, SFMI01, and CAG-485 being the dominant genera and showing no significant differences among groups. The substitution level of LR showed no linear correlation with the abundance of these dominant genera. Anti-inflammatory effects are typically exhibited by Faecousia, which may attenuate chronic inflammation such as rheumatoid arthritis and inflammatory bowel disease by modulating pro-inflammatory cytokines like IL-17 and by secreting anti-inflammatory molecules [48]. Genomic analysis indicates that *CAG-485* encodes glycoside hydrolase family 97 proteins, which may participate in the degradation of carbohydrates like glucans. Combined with the robust polysaccharide metabolic capacity of its affiliated family, Muribaculaceae, it is hypothesized that CAG-485 assists in breaking down complex polysaccharides in the gut, providing a foundation for subsequent metabolic processes such as short-chain fatty acid production [49].

Alpha diversity describes the variety within a given environment or ecosystem and mainly indicates species richness and evenness [50]. While no significant differences in cecal microbiota alpha diversity indices were detected across groups, the Chao1 and Observed_species indices exhibited a linear upward trend. This suggests that LR helps increase the species richness of the cecal microbiota, a trend similar to data observed in weaned piglets in a prior investigation [51]. Through the application of unweighted and weighted UniFrac distances, Principal Coordinates Analysis (PCoA) revealed discrepancies in the beta diversity profile of the cecal microbiome. The distinct clustering and separation of the 15% LR group indicate that a low substitution level of LR has a modulating effect, whereas higher levels (≥30%) imposed a selective pressure, allowing only a minority of tolerant bacteria to thrive, leading to convergent community structures. This observation aligns with the theory "beta diversity reflects differences in species composition between communities" proposed by Jost [52].

4. Conclusion

This study demonstrated that substituting ryegrass with LR in the diet of rabbits significantly enhanced the ADFI and ADG, reduced the F/G, improved slaughter performance, elevated antioxidant capacity and modulated the diversity of the cecal microbiota. Overall, the 15% LR substitution level is recommended as optimal, as it enhances production efficiency without inducing negative effects on the rabbits.

Author Contributions: writing—original draft preparation, X.R. and Q.W.; data curation, X.R. and Q.W.; writing—review and editing, M.Z., J.W and S.Z.; formal analysis, X.R. and J.W.; methodology, M.Z., Q.W.and J.W.; Conceptualization, S.Z. and J.W.; funding acquisition, S.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Xinjiang Pishan Meat Rabbit Science and Technology Backyard, grant number S2025H41280 and the Tarim University Graduate Innovation and Entrepreneurship Program, grant number TDGRI202338.

Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee of Tarim University (Approval Number: PA20250620001, 20 June 2025).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data generated in this study have been deposited in the NCBI database with accession PRJNA1393625 (https://www.ncbi.nlm.nih.gov/).

 doi:10.20944/preprints202605.1776.v1

Acknowledgments: The authors sincerely thank the other members of their research group for providing technical assistance. We also thank Tarim University for providing relevant equipment and facility support for this research.

Conflicts of Interest: The authors declare no conflicts of interest.

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