Effects of Rumen-Protected Lysine on Rumen Microbial Protein Production, Milk Performance, and Nitrogen Excretion in Dairy Cows: Postprint

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Abstract

This study aimed to investigate the effects of rumen-protected lysine (RPLys) on rumen microbial protein (MCP) production, lactation performance, and nitrogen excretion in dairy cows. Forty Holstein dairy cows with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90±15) d] and in good body condition were selected and randomly allocated to four groups (n=10). The control, treatment 1, treatment 2, and treatment 3 groups received dietary supplementation of 0, 25, 30, and 35 g/(d · head) RPLys, respectively. The experiment consisted of a 15-day preliminary period followed by a 60-day formal experimental period. The results showed that: 1) Rumen MCP yield in treatments 1, 2, and 3 increased by 5.34% ($P<0.05$), 14.76% ($P<0.01$), and 10.06% ($P<0.01$) compared with the control group, respectively. 2) Milk yield in treatments 1, 2, and 3 increased by 5.34% ($P<0.05$), 9.30% ($P<0.01$), and 6.69% ($P<0.05$) compared with the control group, respectively; for milk protein percentage, treatment 2 was significantly higher than the control group ($P<0.01$), and treatment 3 was significantly higher than the control group ($P<0.05$). 3) Total nitrogen excretion in treatments 1, 2, and 3 decreased by 5.70% ($P<0.01$), 9.98% ($P<0.01$), and 7.87% ($P<0.01$) compared with the control group, respectively. It can be concluded that dietary supplementation of RPLys in dairy cows can increase rumen MCP production, reduce total nitrogen excretion, and improve lactation performance. Based on comprehensive consideration of these indicators, the optimal supplementation level of RPLys in dairy cow diets under the conditions of this experiment is 30 g/(d · head).

Full Text

Effects of Rumen-Protected Lysine on Ruminal Microbial Protein Production, Milk Performance and Nitrogen Excretion of Dairy Cows

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Abstract: This experiment was conducted to investigate the effects of rumen-protected lysine (RPLys) on ruminal microbial protein (MCP) production, milk performance, and nitrogen excretion of dairy cows. Forty healthy Holstein lactating cows with similar age, body weight, parity, milk yield, milk composition, and lactation period [(90±15) d] were randomly divided into 4 groups with 10 cows per group. The control group and test groups 1, 2, and 3 received dietary supplementation of 0, 25, 30, and 35 g/(d · head) RPLys, respectively. The pre-trial period lasted 15 days, and the formal trial period lasted 60 days. The results showed: 1) Ruminal MCP production in test groups 1, 2, and 3 increased by 5.34% ($P<0.05$), 14.76% ($P<0.01$), and 10.06% ($P<0.01$) compared with the control group, respectively. 2) Milk yield in test groups 1, 2, and 3 increased by 5.34% ($P<0.05$), 9.30% ($P<0.01$), and 6.69% ($P<0.05$) compared with the control group, respectively; milk protein percentage in test group 2 was extremely significantly higher than that in the control group ($P<0.01$), and test group 3 was significantly higher than the control group ($P<0.05$). 3) Total nitrogen excretion in test groups 1, 2, and 3 decreased by 5.70% ($P<0.01$), 9.98% ($P<0.01$), and 7.87% ($P<0.01$) compared with the control group, respectively. These findings indicate that dietary RPLys supplementation can enhance ruminal MCP production, reduce nitrogen excretion, and improve milk performance of dairy cows. Based on comprehensive consideration of these indicators, the optimal supplemental level of RPLys in dairy cow diets under the present experimental conditions is 30 g/(d · head).

Keywords: rumen-protected lysine; dairy cows; ruminal microbial protein; milk performance; nitrogen excretion

Introduction

In recent years, with the continuous expansion of dairy farming scale in China, the demand for protein feedstuffs such as soybeans has increased substantially. Under intensive farming systems, large amounts of unused nitrogen are directly

excreted through feces and urine, causing both waste of protein resources and environmental pollution. The shortage of protein feed ingredients and environmental pollution have become important factors restricting the development of China' s dairy industry. In practical production, improving dietary protein utilization and reducing nitrogen excretion without affecting milk performance is of great significance for mitigating environmental pollution from dairy farming.

In ruminant protein nutrition, limiting amino acids and dietary amino acid composition patterns are crucial factors determining the efficiency of nitrogen utilization in animals [1]. As the first or second limiting amino acid in ruminants, lysine has been shown to improve dietary protein utilization when rumen-protected and added to ruminant diets, avoiding degradation in the rumen and increasing lysine availability in the small intestine [2]. Wang et al. [3] found that dietary RPLys supplementation significantly increased milk yield and milk protein percentage while improving nitrogen utilization in dairy cows. RPLys can meet the requirement for limiting amino acids, increase the amount of absorbable amino acids in the small intestine, and enhance milk performance. Ouyang [4] reported that lysine supplementation improved organic matter digestibility, increased nitrogen retention, and enhanced nitrogen utilization in lambs. Liu et al. [5] demonstrated that RPLys supplementation in dairy cow diets could balance the amino acid utilization system, promote protein digestion and absorption, improve feed protein utilization and milk yield, and reduce nitrogen excretion.

The essence of protein nutrition is amino acid nutrition, and changes in protein digestibility indirectly reflect changes in amino acid digestibility [6]. Previous research on RPLys in ruminants has primarily focused on milk performance, while studies on the effects of dietary RPLys supplementation on ruminal microbial protein (MCP) production and nitrogen excretion in dairy cows are extremely scarce. This experiment investigated the effects of different levels of RPLys supplementation on ruminal MCP production, milk performance, and nitrogen excretion to determine the optimal supplemental level, aiming to improve milk performance, dietary protein utilization, and ruminal MCP production while reducing nitrogen excretion and improving the farming environment, thereby providing a reference for the healthy and sustainable development of China' s dairy industry.

Materials and Methods

1.1 Experimental Design

The RPLys used in this experiment (70% rumen bypass rate) was provided by Qingdao Runbot Biological Technology Co., Ltd. as white granular material composed of L-lysine hydrochloride, palm oil, and silicon dioxide, containing 50% lysine and 12% moisture.

The experiment adopted a single-factor randomized design. Forty healthy Holstein lactating cows from Yantai Hemuyuan Dairy Farm with similar age, body

weight, parity, milk yield, milk composition, and lactation period [(90±15) d] were randomly divided into 4 groups with 10 cows per group. The control group and test groups 1, 2, and 3 received dietary supplementation of 0, 25, 30, and 35 g/(d · head) RPLys, respectively. Each cow was reserved 0.5 kg wheat bran daily from the diet, which was mixed thoroughly with the RPLys and divided into two equal portions fed twice daily with total mixed ration (TMR). The composition and nutrient levels of TMR are shown in Table 1 .

Table 1 Composition and nutrient levels of the TMR (DM basis) %

Items	Content
Ingredients	
Corn	
Steam-flaked corn	
Wheat bran	
Soybean meal	
Corn DDGS	
Soybean hull	
Whole cottonseed	
Extruded soybean	
Whole-plant corn silage	
Brewer' s grains	
Alfalfa hay	
Chinese wide rye	
Rumen protected fat[1]	
NaCl	
NaHCO	
Premix[2]	
Biological mycotoxin removement agent[3]	
Total	
Nutrient levels	
CP	
NEL/(MJ/kg)	
NDF	
ADF	

[1] Main components of rumen protected fat: palmitic acid 75%, myristic acid 1%-5%, stearic acid 6%-8%, oleic acid 10%, furoic acid 2%.

[2] The premix provided the following per kg of the DM for TMR: VA 8,000 IU, VD 1,600 IU, VE 30 mg, Fe 20 mg, Cu 16 mg, Zn 100 mg, Mn 35 mg, I 1 mg, Se 0.5 mg, Co 0.5 mg.

[3] Main components of biological mycotoxin removement agent: mannan oligosaccharide 14%, -glucan 15%-40%, crude protein 35%, moisture 6%.

NEL was a calculated value which was the sum of NEL of different ingredients multiplied by their percentages in the TMR [7], while the other nutrient levels were measured values.

1.2 Management

Cows were fed in separate pens throughout the 75-day experimental period, including a 15-day pre-trial period and a 60-day formal trial period. Cows were milked three times daily (04:00, 12:00, 18:00) using imported Dutch SAC automatic milking machines and fed TMR twice daily (04:30, 18:30), ensuring cows had access to TMR for more than 20 hours per day. After feeding, cows were allowed free movement and drinking water in the exercise yard, and were managed according to routine deworming, lighting, and management protocols.

1.3 Sample Collection

1.3.1 TMR Samples

TMR samples were collected three times using the quartering method on days 1–3 of the pre-trial period, days 28–30 of the formal trial period, and days 58–60 of the formal trial period. Collected TMR samples were dried at 65°C in a constant temperature oven to produce air-dried samples, which were then crushed and mixed for storage.

1.3.2 Urine Samples

Urine samples were collected on days 1–3 of the pre-trial period, days 28–30 of the formal trial period, and days 58–60 of the formal trial period. Following the spot urine collection method described by Zhu [6], urine was collected twice daily at 12-hour intervals using manual collection combined with bladder catheterization for three consecutive days, with collection delayed by 4 hours each day compared to the previous day. Ten percent sulfuric acid was added to each urine sample to adjust pH (pH<3), and samples were frozen at -20°C for storage.

1.3.3 Fecal Samples

Fecal samples were collected three times on days 1–3, days 28–30, and days 58–60 of the formal trial period, with total fecal collection for 24 hours over three consecutive days. Before each collection, cow beds were thoroughly washed, and feces were collected promptly into buckets. Daily fecal samples were mixed evenly and weighed, and quartering was used to collect daily feces. Nitrogen fixation was performed by adding 25 mL of 10% sulfuric acid per 100 g of feces, and samples were frozen at -20°C. After each sampling period, three-day fecal samples were mixed proportionally by weight, dried to constant weight at 65°C in a constant temperature oven, and stored as air-dried samples.

1.3.4 Milk Samples

Milk samples (50 mL) were collected on day 1 of the pre-trial period and every 15 days during the formal trial period at a ratio of 4:3:3 for morning, afternoon, and evening milking. Samples were preserved with 30 mg potassium dichromate, mixed evenly, and refrigerated at 4°C for determination of milk composition indices.

1.4 Measurements

1.4.1 Feed Intake

Cows were fed in separate pens, and feed intake was recorded individually. During the pre-trial period, feed amounts were recorded every 2 days, with leftover feed collected and weighed before each feeding to calculate individual intake. Using the same method during the formal trial period, intake was recorded and calculated every 10 days for a total of 6 recordings, each over 3 consecutive days, to determine average intake for each period. TMR feeding amounts for the next period were adjusted based on the average intake measured in the previous period.

1.4.2 Routine Nutrient Contents

Moisture content was determined according to GB/T 6435-2006 [8] to calculate dry matter (DM) content. Crude protein (CP) content was determined by the Kjeldahl method (GB/T 6432-1994 [9]). Neutral detergent fiber (NDF) content was determined according to GB/T 20806-2006 [10]. Acid detergent fiber (ADF) content was determined according to NY/T 1459-2007 [11]. Calcium (Ca) content was determined by the potassium permanganate method (GB/T 6436-2002 [12]). Phosphorus (P) content was determined by spectrophotometry (GB/T 6437-2002 [13]).

1.4.3 Ruminal MCP Production

Purine derivatives (PD) excreted in urine mainly originate from rumen microbial purines; therefore, ruminal MCP production can be estimated by measuring urinary PD content. Urinary uric acid and allantoin contents were determined using colorimetric methods, with the sum representing urinary PD content [14]. Uric acid was measured using a UV-1800 PC spectrophotometer (Shanghai Mapada Instruments Co., Ltd.), and allantoin was measured using a DNM-9602 microplate analyzer (Beijing Perlong New Technology Co., Ltd.).

The calculation formula for exogenous purine absorption in the small intestine (X) is:

$$Y = 0.85X + 0.385BW^{0.75}$$

Where: Y is urinary PD excretion (mmol/d); 0.85 is the recovery rate of absorbed purines converted to urinary PD in cattle; 0.385 is endogenous PD excretion in urine when purine absorption is zero; BW · is metabolic body weight (kg).

The calculation formula for ruminal MCP production is:

$$MCP(g/d) = \frac{6.25 \times 70X}{0.83 \times 0.116 \times 1000} = 6.25 \times 0.727X$$

Where: X is exogenous purine absorption in the small intestine (mmol/d); 70 is the nitrogen content per mole of purine (mg/mol); 0.83 is the digestibility of microbial nucleic acid purines; 0.116 is the proportion of purine nitrogen in total microbial nitrogen; 6.25 is the average coefficient for converting nitrogen to protein.

1.4.4 Milk Yield and Composition

Milk yield was automatically displayed during milking using the Dutch SAC automatic milking machine and recorded every 5 days during both pre-trial and formal trial periods, with each recording over 3 consecutive days to obtain a 3-day average. Milk composition including fat percentage, protein percentage, lactose percentage, and somatic cell count was determined using an automatic milk composition and somatic cell analyzer (Combi Foss FT+, Foss, Denmark) at the Dairy Performance Testing Laboratory of Shandong Academy of Agricultural Sciences, and average values were calculated for the formal trial period.

1.4.5 Nitrogen Metabolism Indices

Urinary urea nitrogen was determined by the urease method [15], and urinary creatinine was determined by the picric acid colorimetric method [16], with reagent kits purchased from Nanjing Jiancheng Bioengineering Institute. Following Valadares et al. [16], urinary volume was determined using creatinine as a marker (approximately 29 mg creatinine excreted per kg body weight per day). A UV-1800 PC spectrophotometer (Shanghai Mapada Instruments Co., Ltd.) was used for these determinations.

Nitrogen metabolism indices were calculated as follows:

- Fecal nitrogen (g/d) = daily nitrogen excretion × CP content in feces × 0.16
- Urinary nitrogen (g/d) = daily urinary volume × urinary nitrogen content
- Milk nitrogen (g/d) = milk yield × milk protein percentage × 0.16
- Digestible nitrogen (g/d) = dietary nitrogen intake - fecal nitrogen
- Total nitrogen excretion (g/d) = fecal nitrogen + urinary nitrogen
- Nitrogen retention (g/d) = dietary nitrogen intake - fecal nitrogen - urinary nitrogen - milk nitrogen
- Nitrogen apparent digestibility (%) = [(dietary nitrogen intake - fecal nitrogen) / dietary nitrogen intake] × 100

1.5 Data Processing and Analysis

Experimental data were preliminarily processed using Excel 2010 software. One-way ANOVA was performed using SPSS 20.0 software, and Duncan' s multi-

ple comparison test was used to examine significant differences among groups. P<0.05 and P<0.01 were considered significant and extremely significant, respectively. Results are expressed as means ± standard error.

Results

2.1 Effects of RPLys Supplemental Level on Ruminal MCP Production of Dairy Cows

As shown in Table 2 , urinary uric acid excretion in all test groups was extremely significantly higher than in the control group (P<0.01), with the 30 g/(d · head) RPLys group being extremely significantly higher than the 25 g/(d · head) group (P<0.01) but not significantly different from the 35 g/(d · head) group (P>0.05). Urinary allantoin excretion in the 30 and 35 g/(d · head) RPLys groups was extremely significantly higher than in the control group (P<0.01), while the 25 g/(d · head) group showed no significant difference from the control group (P>0.05); the 30 g/(d · head) group was also extremely significantly higher than the 25 g/(d · head) group (P<0.01). Urinary PD excretion in the 25 g/(d · head) RPLys group was significantly higher than in the control group (P<0.05), while the 30 and 35 g/(d · head) groups were extremely significantly higher than the control group (P<0.01); the 30 g/(d · head) group was extremely significantly higher than the 25 g/(d · head) group (P<0.01) but not significantly different from the 35 g/(d · head) group (P>0.05). Ruminal MCP production in the 25 g/(d · head) RPLys group was significantly higher than in the control group (P<0.05), while the 30 and 35 g/(d · head) groups were extremely significantly higher than the control group (P<0.01); the 30 g/(d · head) group was extremely significantly higher than the 25 g/(d · head) group (P<0.01) but not significantly different from the 35 g/(d · head) group (P>0.05). Ruminal MCP production in the 25, 30, and 35 g/(d · head) RPLys groups increased by 5.34%, 14.76%, and 10.06% compared with the control group, respectively.

Table 2 Effects of RPLys supplemental level on ruminal MCP production of dairy cows

Items	RPLys supplemental level/[g/(d · head)]			
	0	25	30	35
Uric acid/(mmol/d)	34.19±2.44Cc	40.50±1.10ABab		
Allantoin/(mmol/d)	279.60±2.09Cc	290.04±4.55ABab		
Urinary PD/(mmol/d)	313.79±4.06Cc	330.55±5.09ABab		
Ruminal MCP production/(g/d)	1,425.79±18.44Cc	1,501.92±23.14ABab		

In the same row, values with different small letter superscripts mean significant difference (P<0.05), and with different capital letter superscripts mean extremely significant difference (P<0.01), while with the same or no letter superscripts mean no significant difference (P>0.05). The same as below.

2.2 Effects of RPLys Supplemental Level on DMI and Milk Performance of Dairy Cows

As shown in Table 3 , dry matter intake (DMI) showed no significant differences among all test groups compared with the control group (P>0.05). During the formal trial period, milk yield in the 25 and 35 g/(d · head) RPLys groups was significantly higher than in the control group (P<0.05), while the 30 g/(d · head) group was extremely significantly higher than the control group (P<0.01); milk yield in the 25, 30, and 35 g/(d · head) RPLys groups increased by 5.34%, 9.30%, and 6.69% compared with the control group, respectively. Milk protein percentage in the 30 g/(d · head) RPLys group was extremely significantly higher than in the control group (P<0.01), and the 35 g/(d · head) group was significantly higher than the control group (P<0.05), while the 25 g/(d · head) group showed no significant difference from the control group (P>0.05). No significant differences were observed among any test groups compared with the control group for milk fat percentage, lactose percentage, or milk somatic cell count (P>0.05).

Table 3 Effects of RPLys supplemental level on DMI and milk performance of dairy cows

Items	RPLys supplemental level/[g/(d · head)]			
	0	25	30	35
DMI/(kg/d)	21.70±0.14	21.75±0.11	21.86±0.08	21.78±0.12
Milk yield/(kg/d) (Preliminary)	23.36±0.69	23.41±0.73	23.79±0.45	23.45±0.39
Milk yield/(kg/d) (Trial)	23.77±0.24Bb	25.04±0.78ABab	25.96±0.36Aa	25.20ABa
Milk fat percentage/% (Preliminary)	3.88±0.06	3.89±0.06	3.90±0.05	3.90±0.07
Milk fat percentage/% (Trial)	3.91±0.05	3.94±0.08	3.90±0.03	3.98±0.13

Items	RPLys supplemental level/[g/(d · head)]		
Milk protein percentage/% (Preliminary)	3.28±0.03	3.29±0.03	3.30±0.05
Milk protein percentage/% (Trial)	3.30±0.03Bc	3.36±0.02ABab	
Lactose percentage/% (Preliminary)	4.47±0.07	4.48±0.06	4.49±0.08
Lactose percentage/% (Trial)	4.46±0.06	4.45±0.02	4.45±0.06
Milk somatic cell count/×10³ (Preliminary)	175.17±3.19	176.63±3.88	174.88±3.96
Milk somatic cell count/×10³ (Trial)	165.94±2.75	160.44±3.23	

2.3 Effects of RPLys Supplemental Level on Nitrogen Excretion and Nitrogen Apparent Digestibility of Dairy Cows

As shown in Table 4 , nitrogen intake showed no significant differences among all test groups compared with the control group (P>0.05). Fecal nitrogen and urinary nitrogen excretion in all test groups were extremely significantly lower than in the control group (P<0.01), with urinary nitrogen excretion in the 30 g/(d · head) RPLys group being extremely significantly lower than in the 25 g/(d · head) group. Milk nitrogen excretion in all test groups was extremely significantly higher than in the control group (P<0.01), with the 30 g/(d · head) RPLys group being extremely significantly higher than both the 25 and 35 g/(d · head) groups (P<0.01). Digestible nitrogen in all test groups was extremely

significantly higher than in the control group (P<0.01), with the 30 g/(d · head) group being significantly higher than the 25 g/(d · head) group (P<0.05) but not significantly different from the 35 g/(d · head) group (P>0.05). Total nitrogen excretion in all test groups was extremely significantly lower than in the control group (P<0.01), with the 30 g/(d · head) group being extremely significantly lower than the 25 g/(d · head) group (P<0.01); total nitrogen excretion in the 25, 30, and 35 g/(d · head) RPLys groups decreased by 5.70% (P<0.01), 9.98% (P<0.01), and 7.87% (P<0.01) compared with the control group, respectively. Nitrogen retention in all test groups was extremely significantly higher than in the control group (P<0.01). Nitrogen apparent digestibility in all test groups was extremely significantly higher than in the control group (P<0.01).

Table 4 Effects of RPLys supplemental level on nitrogen excretion and nitrogen apparent digestibility of dairy cows

Items	RPLys supplemental level/[g/(d · head)]				
	0	25	30	35	
Intake N/(g/d)	542.88±1.90	544.77	5	5	5±0.95
Feces N/(g/d)	167.50±3.53Aa	154.84	1	1	1.44Bb
Urine N/(g/d)	239.77±2.50Aa	229.23	2	2	2.41BCbc
Milk N/(g/d)	125.41±1.07Cc	134.71	1	1	0.95Bb
Digestible N/(g/d)	375.39±2.14Bc	389.94	3	3	1.77Aab
N total excretion/(g/d)	407.27±2.83Aa	384.06	3	3	1.22BCbc
N deposition/(g/d)	10.20±2.26Bb	26.00	3	3	1.52Aa
N apparent digestibility/%	69.26±0.58Bb	71.63	7	7	0.27Aa

Discussion

3.1 Effects of RPLys Supplemental Level on Ruminal MCP Production of Dairy Cows

Urinary PD content is highly correlated with ruminal MCP production [17]. Since PD excreted in urine mainly originate from rumen microbial purines, ru-

minal MCP production can be estimated by measuring urinary PD content. Under the conditions of this experiment, supplementation with different levels of RPLys significantly or extremely significantly increased ruminal MCP production in dairy cows. Liu [18] reported that lysine supplementation increased total volatile fatty acids, acetate, propionate, and butyrate concentrations in rumen fluid, increased total bacterial count, and improved apparent digestibility of organic matter and crude protein, thereby improving rumen fermentation patterns, enhancing rumen digestive metabolism, and increasing ruminal MCP production in sheep. Huang [6] found that dietary lysine increased allantoin and PD excretion, indirectly promoting ruminal MCP production in sika deer. Lin et al. [19] reported that rumen-protected lysine supplementation significantly increased ruminal MCP production in sheep. Changes in rumen NH -N concentration reflect the rate of dietary nitrogen degradation by rumen microorganisms and the utilization rate of ammonia nitrogen (NH -N) by rumen microorganisms, which indirectly affects MCP production. Dietary RPLys supplementation improved rumen fermentation patterns, resulting in a MCP synthesis rate greater than the protein decomposition rate to NH -N, thereby increasing ruminal MCP production. In this experiment, ruminal MCP production increased initially and then decreased with increasing RPLys supplementation, with the most pronounced increase at 30 g/(d·head). This may be due to amino acid antagonism [20]; high-dose lysine supplementation may interfere with the absorption and metabolism of other amino acids, preventing further increases in ruminal MCP production.

3.2 Effects of RPLys Supplemental Level on DMI and Milk Performance of Dairy Cows

Robinson et al. [21] found that RPLys supplementation in lactating dairy cow diets had no significant effect on DMI. Han et al. [22] also reported that RPLys supplementation had no significant effect on DMI in Holstein dairy cows. Under the conditions of this experiment, dietary RPLys supplementation had no significant effect on DMI in Holstein dairy cows, consistent with the above studies. Yun [23] found that rumen-protected lysine supplementation had no significant effect on DMI but significantly increased milk yield and milk protein content. RPLys can meet the requirement for limiting amino acids in lactating cows, increase the amount of absorbable amino acids in the small intestine, and thereby improve production performance. Giallongo et al. [24] found that RPLys infusion increased milk yield, promoted milk protein synthesis, and improved milk protein percentage. Liu et al. [5] reported that dietary RPLys supplementation reduced the requirement for rumen-undegradable protein, met the requirement for limiting amino acids, and improved milk yield and milk protein percentage. Tang et al. [25] found that RPLys supplementation significantly increased milk protein percentage in lactating buffaloes. Giallongo et al. [26] reported that RPLys improved milk yield and milk protein percentage in dairy cows. Intestinally digestible crude protein (IDCP) in dairy cow diets is the source for milk protein synthesis [27]; RPLys supplementation increased total IDCP

and improved the composition ratio of digestible amino acids in IDCP, thereby increasing milk protein content. In this experiment, dietary RPLys supplementation significantly improved milk protein percentage, likely by improving amino acid composition in the small intestine and enhancing protein utilization. Milk yield and milk protein percentage increased initially and then decreased with increasing RPLys supplementation, with the most pronounced improvement at 30 g/(d · head). Changes in milk fat percentage, lactose percentage, and somatic cell count were not obvious. The reduction in milk yield and milk protein percentage at high RPLys doses may be due to antagonism between lysine and other amino acids, affecting their digestion and absorption [20], reducing dietary protein utilization, and consequently decreasing milk yield and milk protein percentage.

3.3 Effects of RPLys Supplemental Level on Nitrogen Excretion and Nitrogen Apparent Digestibility of Dairy Cows

Nitrogen digestion and metabolism reflect dietary protein deposition efficiency, amino acid balance, and are closely related to animal performance. Dietary RPLys supplementation can improve protein digestion and absorption, enhance protein utilization, and reduce nitrogen excretion [28]. Ammonia, as a product of dietary protein degradation, is the primary nitrogen source for rumen microbial growth. Mao [29] reported that lysine reduced plasma urea nitrogen content, improved protein digestion and absorption, and increased nitrogen deposition rate. Under the conditions of this experiment, dietary RPLys supplementation extremely significantly reduced fecal nitrogen, urinary nitrogen, and total nitrogen excretion while significantly increasing digestible nitrogen and nitrogen apparent digestibility. Socha et al. [30] found that dietary RPLys supplementation reduced urinary nitrogen concentration, improved nitrogen conversion efficiency, enhanced protein utilization, and decreased nitrogen excretion. Fang et al. [31] reported that lysine reduced urea nitrogen concentration and significantly decreased fecal nitrogen excretion while improving nitrogen apparent digestibility in lactating sows. Li et al. [32] found that dietary RPLys supplementation improved dietary protein conversion efficiency, nitrogen retention, and nitrogen apparent digestibility in weaned lambs. Whelan et al. [33] reported that dietary lysine improved amino acid balance, reduced urinary nitrogen emission, and enhanced nitrogen utilization. RPLys supplementation increased total intestinally digestible amino acids, reduced losses during protein transformation in the rumen, and further improved dietary protein utilization, thereby decreasing nitrogen excretion. Rumen nitrogen metabolism is closely related to MCP production; dietary RPLys supplementation improved rumen digestive metabolism, resulting in a MCP synthesis rate greater than the protein decomposition rate to NH -N, increasing ruminal MCP production, reducing NH -N losses, improving nitrogen utilization, and decreasing total nitrogen excretion. In this experiment, total nitrogen excretion decreased initially and then increased with increasing RPLys supplementation, with the most pronounced reduction at 30 g/(d · head). The relative increase in total nitrogen excretion at high RPLys doses may be

due to antagonism between lysine and arginine, which share the same membrane transport carrier; high lysine supplementation may affect arginine absorption, increase arginine degradation in the kidneys, and increase urinary arginine and urea excretion [27], thereby increasing urinary nitrogen excretion and total nitrogen excretion in high-dose groups.

Conclusion

Dietary RPLys supplementation can increase ruminal MCP production and milk performance while reducing nitrogen excretion in dairy cows. Based on comprehensive consideration of these indicators, the optimal supplemental level of RPLys in dairy cow diets under the present experimental conditions is 30 g/(d·head).

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