Effects of Rumen-Protected Methionine and Lysine Supplementation on Growth Performance and Carcass Chemical Composition in Holstein Dairy Bulls (Postprint)

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Abstract

This study aimed to investigate the effects of dietary supplementation with rumen-protected methionine (RPMet) and rumen-protected lysine (RPLys) on growth performance and carcass chemical composition in Holstein dairy bulls, and to explore the feasibility of using RPMet and RPLys to replace partial dietary crude protein. A completely randomized design was employed, utilizing twenty-five healthy Holstein dairy bulls at approximately 12 months of age with similar body weight and body measurements, which were randomly assigned to five groups (n = 5 per group). The control group received a basal diet, while the four experimental groups (M15, L30, M15+L30, and LCP) were supplemented with RPMet 15 g/d, RPLys 30 g/d, RPMet 15 g + RPLys 30 g/d, and RPMet 15 g + RPLys 30 g/d (with dietary crude protein reduced by 2.20%), respectively, in addition to the basal diet. The experiment consisted of a 10-day preliminary period followed by a 150-day experimental period. The results demonstrated that, compared with the control group: 1) Dietary supplementation with RPMet and RPLys had no significant effect on dry matter intake (DMI) in dairy bulls ($P > 0.05$); the final body weight (FBW) and average daily gain (ADG) of the L30, M15+L30, and LCP groups were significantly increased ($P < 0.05$), with the highest values observed in the M15+L30 group, though no significant difference existed between the M15+L30 and LCP groups ($P > 0.05$); the feed conversion ratio (FCR) of the M15+L30 and LCP groups was significantly decreased ($P < 0.05$), with the M15+L30 group showing the lowest value, and the difference between these two groups was not significant ($P > 0.05$). 2) Chest circumference and carcass crude protein content in dairy bulls of the M15+L30 and LCP groups were significantly increased ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$). 3) The contents of essential amino acids

(threonine, leucine, and arginine) in the carcass of dairy bulls in the M15+L30 and LCP groups were significantly increased ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$), while the isoleucine content in the M15+L30 group was significantly increased ($P < 0.05$); the contents of non-essential amino acids (aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, and proline) and total amino acids in the carcass of dairy bulls in the M15+L30 and LCP groups were significantly increased ($P < 0.05$), and the tyrosine content in the L30 and M15+L30 groups was significantly increased ($P < 0.05$), with no significant differences between these two groups ($P > 0.05$). In conclusion, dietary supplementation with RPMet and RPLys improved the growth performance of dairy bulls to a certain extent, increased protein synthesis and amino acid deposition in the carcass, and supplementation with RPMet 15 g + RPLys 30 g/d could partially replace dietary crude protein (2.20%).

Full Text

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Abstract

This study investigated the effects of dietary supplementation with rumen-protected methionine (RPMet) and rumen-protected lysine (RPLys) on growth performance and carcass chemical composition of Holstein bulls, and explored the feasibility of using RPMet and RPLys to replace a portion of dietary crude protein. A completely randomized design was employed with twenty-five healthy Holstein bulls aged approximately 12 months and similar in body weight and measurements, randomly allocated into five groups of five animals each. The control group received a basal diet, while four experimental groups (M15, L30, M15+L30, and LCP) received the basal diet supplemented with RPMet 15 g/d, RPLys 30 g/d, RPMet 15 g + RPLys 30 g/d, or RPMet 15 g + RPLys 30 g/d (with simultaneous reduction of dietary crude protein by 2.20%), respectively. The adaptation period lasted 10 days, followed by a 150-day experimental period.

The results showed that, compared with the control group: (1) supplementation with RPMet and RPLys had no significant effect on dry matter intake ($P > 0.05$); final body weight and average daily gain were significantly increased in the L30, M15+L30, and LCP groups ($P < 0.05$), with the highest values observed in the M15+L30 group, though not significantly different from the LCP group (P > 0.05); feed-to-gain ratio was significantly reduced in the M15+L30 and LCP groups (P < 0.05), with the M15+L30 group showing the lowest value, but the difference between these two groups was not significant (P > 0.05). (2) Chest girth and carcass crude protein content were significantly increased in the M15+L30 and LCP groups (P < 0.05), with no significant difference between these two groups (P > 0.05). (3) The M15+L30 and LCP groups showed significantly higher contents of essential amino acids threonine, leucine, and arginine (P < 0.05), with no significant difference between them (P > 0.05); the M15+L30 group showed significantly higher isoleucine content (P < 0.05). For non-essential amino acids, the M15+L30 and LCP groups exhibited significantly higher contents of aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, and proline, as well as total amino acid content (P < 0.05); the L30 and M15+L30 groups showed significantly higher tyrosine content (P < 0.05), with no significant difference between them (P > 0.05). It is concluded that dietary supplementation with RPMet and RPLys can improve growth performance and increase protein synthesis and amino acid deposition in carcasses of Holstein bulls, and that supplementation with RPMet 15 g + RPLys 30 g/d can partially replace dietary crude protein (2.20%).

Keywords: rumen-protected methionine; rumen-protected lysine; Holstein bulls; growth performance; carcass chemical composition

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Introduction

Methionine (Met) and lysine (Lys) are the primary limiting amino acids in metabolizable protein for ruminants and represent the first or second limiting amino acids during animal growth. Research on limiting amino acids promotes improved amino acid balance of metabolizable protein, enhanced utilization efficiency of protein feedstuffs, and reduced nitrogen excretion in ruminant manure and urine, which holds significant importance for developing an economically efficient and environmentally friendly society. Consequently, studies on amino acid nutrition, particularly limiting amino acids, have become essential and central to protein nutrition research in ruminants [1]. Rumen-protected amino acids are modified or protected using specific methods to prevent degradation by ruminal microorganisms [2], thereby improving the balance of absorbable amino acids in the small intestine and enhancing absorption efficiency. Recent domestic and international research on methionine and lysine has primarily focused on supplementation with rumen-protected methionine (RPMet) and rumen-protected lysine (RPLys) to increase milk yield and protein content in dairy cows [3], improve growth performance in beef cattle [4], and reduce dietary protein levels [5]. Ding et al. [4] reported that supplementation with RPMet 30 g/d, RPLys 30 g/d, or a combination of RPLys 30 g/d + RPMet 30 g/d in the basal diet of Charolais cattle increased average daily gain (ADG) by 9%, 6%, and 9% respectively compared with the control group, demonstrating favorable application effects of RPMet and RPLys in beef cattle. With increasing shortage of beef resources and rising beef prices in China, coupled with a surge in dairy cattle numbers, fattening of dairy bulls has become an important utilization method for male dairy calves and a significant beef resource. Guo et al. [6] reported that increasing dietary protein and energy levels in dairy bulls improved meat-to-bone ratio and net meat yield while enhancing meat quality. Therefore, nutritional regulation to improve growth performance and meat quality of dairy bulls has emerged as a new challenge in beef cattle production. Currently, no domestic reports have documented the application of RPMet and RPLys in dairy bulls.

The present study utilized 12-month-old dairy bulls to investigate the effects of RPMet, RPLys, and their combination on growth performance and carcass chemical composition, explore the feasibility of using RPMet and RPLys to replace a portion of dietary crude protein (CP), and provide a theoretical basis for the application of RPMet and RPLys in dairy bulls.

Materials and Methods

Experimental Animals and Design

A completely randomized design was employed with twenty-five healthy Holstein bulls aged approximately 12 months and similar in body weight and measurements, randomly allocated into five groups of five animals each. The control group received a basal diet, while four experimental groups received the basal diet supplemented with RPMet 15 g/d (M15 group), RPLys 30 g/d (L30 group), RPMet 15 g/d + RPLys 30 g/d (M15+L30 group), or RPMet 15 g/d + RPLys 30 g/d (with simultaneous reduction of dietary CP by 2.20%) (LCP group). The adaptation period lasted 10 days, followed by a 150-day experimental period. Basic information on the experimental animals is presented in Table 1 .

Experimental Diets

RPMet and RPLys were purchased from Beijing Yahe Co., Ltd., with rumen protection rates of 60.25% and 68.81%, respectively. Roughage consisted of Chinese wildrye. The basal diet was formulated according to the "Feeding Standard of Beef Cattle"(NY/T 815-2004) based on the nutritional requirements for a 300-kg beef cattle with a daily gain of 1 kg/d. The composition and nutrient levels of the basal diet are shown in Table 2 .

Feeding Management

Following uniform deworming, all experimental bulls were housed in the same barn and individually tethered in separate stalls. Animals were fed twice daily at 08:00 and 16:00. The concentrate allowance was adjusted once every two weeks based on body weight and feed intake. Roughage and concentrate were fed separately using a roughage-first feeding method. Water was provided 0.5 hours after feeding.

Sample and Data Collection

Diet samples were collected on the final day of the adaptation period and on days 50, 100, and 150 of the experimental period using the quartering method, with 1 kg of concentrate and roughage samples collected each time for determination of nutrient contents. Body weight and measurements were recorded at the beginning and end of the experiment (fasting weight measured at 07:00). Beef samples were collected after a 24-hour fasting period pre-slaughter; post-slaughter, carcasses were aged at 0–4°C for 48 hours, and meat samples from the 12th–13th rib eye muscle were collected, skinned, defatted, and de-fascialed before storage at -80°C.

Analytical and Determination Methods

Dietary nutrient contents were determined according to "Feed Analysis and Feed Quality Detection Technology" [8]. Crude protein (CP) content was measured using a FOSS automatic Kjeldahl nitrogen analyzer. Acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined using an ANKOM A2000i (filter bag technique) automatic fiber analyzer. Calcium content was measured by the potassium permanganate method, and phosphorus content by the ammonium vanadomolybdate colorimetric method. Growth performance parameters included body weight measured using a platform scale; body height (vertical distance from the highest point of the withers to the ground), body length (distance from shoulder to ischium) measured using a measuring stick; chest girth (vertical circumference at the posterior angle of the scapula) measured using a tape measure; total weight gain calculated as the difference between final and initial weight; ADG calculated as total weight gain divided by experimental days; feed-to-gain ratio (F/G) calculated as feed consumption per kilogram of weight gain; and daily feed intake recorded to calculate dry matter intake (DMI). Carcass chemical composition was determined according to AOAC methods [9]. Eye muscle samples (500 g) were minced after thawing. Moisture content was determined by vacuum high-temperature drying, CP content by semi-micro Kjeldahl method, crude ash content by combustion gravimetric method, crude fat content by Soxhlet extraction, and amino acid content by Tecator-1030 nitrogen analyzer and Agilent liquid chromatograph.

Statistical Analysis

Data were processed using Excel and analyzed by one-way ANOVA using SPSS 19.0. Duncan' s multiple range test was used for pairwise comparisons. Results were expressed as "mean $\pm$ standard error,"with $P < 0.05$ considered statistically significant.

Results

Weight Gain and Body Measurements

As shown in Table 3 , dietary supplementation with RPMet and RPLys had no significant effect on DMI in dairy bulls ($P > 0.05$). Compared with the control group, final body weight was significantly higher in the L30, M15+L30, and LCP groups ($P < 0.05$), with the M15+L30 group showing the highest value, which was not significantly different from the LCP group ($P > 0.05$) but significantly higher than the L30 group ($P < 0.05$). ADG was significantly higher in the L30, M15+L30, and LCP groups compared with the control group ($P < 0.05$), with no significant difference between the M15+L30 and LCP groups ($P > 0.05$), though both were significantly higher than the L30 group ($P < 0.05$). Feed-to-gain ratio was significantly lower in the M15+L30 and LCP groups compared with the control and M15 groups ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$). Chest girth was significantly higher in the M15+L30 and LCP groups compared with the control group ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$). No significant differences were observed in body height or body length among all groups ($P > 0.05$).

Carcass Chemical Composition

As shown in Table 4 , carcass CP content was significantly higher in the M15+L30 and LCP groups compared with the control group ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$). No significant differences were observed among groups in carcass crude fat, crude ash, or moisture contents ($P > 0.05$).

Carcass Amino Acid Content

As shown in Table 5 , for essential amino acids (EAA), threonine, leucine, and arginine contents were significantly higher in the M15+L30 and LCP groups compared with the control, M15, and L30 groups ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$). Isoleucine content was significantly higher in the M15+L30 group compared with all other groups ($P < 0.05$), with no significant differences among the remaining four groups ($P > 0.05$). For non-essential amino acids (NEAA), aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, and proline contents were significantly higher in the M15+L30 and LCP groups compared with the control, M15, and L30 groups ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$). Tyrosine content was significantly higher in the L30 and M15+L30 groups compared with the other three groups ($P < 0.05$), with no significant difference between these two groups ($P > 0.05$). Total amino acid (TAA) content was significantly higher in the M15+L30 and LCP groups compared with the other three groups ($P < 0.05$). The ratio of EAA to TAA followed the order: control > L30 > M15 > LCP > M15+L30, with no significant differences among groups (P > 0.05).

Discussion

Effects of RPMet and RPLys Supplementation on Weight Gain and Body Measurements of Holstein Bulls

Beef cattle fattening aims to achieve high daily weight gain, quality beef, and improved feed conversion efficiency; therefore, growth performance can be directly reflected by ADG and feed-to-gain ratio. In this study, final body weight and ADG were significantly increased in the L30, LCP, and M15+L30 groups, with the M15+L30 group showing optimal performance, consistent with the report by Ding et al. [4]. Feed-to-gain ratio was significantly reduced in the LCP and M15+L30 groups, with the M15+L30 group showing the lowest value, aligning with the findings of Veira et al. [10]. These improvements may be attributed to methionine and lysine being the primary limiting amino acids in corn-soybean meal-based diets [11-12], and their supplementation improved amino acid balance in the small intestine, preventing absorption disorders of other amino acids caused by limiting amino acid deficiency and thereby enhancing amino acid utilization efficiency. This may explain why the M15+L30 group showed optimal performance in final body weight, ADG, and feed-to-gain ratio. Additionally, since amino acids are the basic functional units of protein, dietary protein level influences the efficacy of rumen-protected amino acid application. Regarding the feasibility of using RPMet and RPLys to replace a portion of dietary protein in dairy bulls, Yun et al. [13] reported that reducing dietary CP from 14.67% to 12.02% and supplementing with RPMet and RPLys resulted in higher body weight gain than the 14.67% CP diet, indicating that RPMet and RPLys could replace part of dietary CP and even improve growth performance. In this study, final body weight and ADG in the LCP group were slightly lower than in the M15+L30 group, and feed-to-gain ratio was slightly higher, with no significant differences between the two groups. This suggests that using RPMet 15 g/d + RPLys 30 g/d to replace part of the dietary CP can improve growth performance in dairy bulls, consistent with Yun et al. [13]. However, Archibeque et al. [14] found that in steers fed RPMet-supplemented concentrate with either high- or low-protein hay, nitrogen retention and ADG were higher in the high-protein hay group at the same RPMet supplementation level, differing from our results. This indicates that the application effects of RPMet and RPLys in beef cattle may be influenced by dietary composition and animal breed.

Body measurements are important quantitative indicators for growth performance evaluation and animal genetic selection, closely associated with many economically important traits [15]. In this study, chest girth was significantly higher in the M15+L30 and LCP groups, possibly related to altered muscle structure proportions in dairy bulls, as neck and shoulder muscle proportions increase with fattening duration. This suggests that supplementation with RP-

Met 15 g/d + RPLys 30 g/d better promoted neck and shoulder muscle growth at this stage, consistent with Owens et al. [16]. Although genetic potential determines animal body measurements, its expression is limited by dietary nutrition. The lack of significant difference in chest girth between the LCP and M15+L30 groups indicates that supplementation with RPMet 15 g/d + RPLys 30 g/d can replace 2.20% of dietary CP while maintaining normal growth and development in dairy bulls.

Effects of RPMet and RPLys Supplementation on Carcass Chemical Composition of Holstein Bulls

The primary nutrients in beef include protein, fat, and moisture. High protein content meets consumer demands for meat products, while appropriate fat levels ensure beef palatability; therefore, nutrient composition is an important criterion for beef quality evaluation. Xue et al. [17] reported that dietary RPLys supplementation in beef cattle resulted in linear reduction of backfat thickness, with 15 g/d lysine significantly decreasing backfat thickness and increasing carcass protein content. This differs from our results, where carcass CP content was not significantly increased in the M15 and L30 groups but was significantly elevated in the M15+L30 and LCP groups, possibly due to differences in supplementation levels, diet types, and cattle breeds. No significant differences were observed among groups in carcass crude fat, crude ash, or moisture contents, consistent with Hussein and Berger [18], who reported that lysine supplementation in high-concentrate diets did not affect carcass quality of Holstein steers. The lack of significant difference in carcass CP content between the LCP and M15+L30 groups, and the absence of significant differences in crude fat, crude ash, or moisture contents among the LCP, control, M15, and L30 groups, indicates that using RPMet 15 g/d + RPLys 30 g/d to replace 2.20% of dietary CP does not affect beef palatability (crude fat) or tenderness (moisture) but enhances muscle protein synthesis, consistent with the improved weight gain observed in this study.

Effects of RPMet and RPLys Supplementation on Carcass Amino Acid Content of Holstein Bulls

As muscle represents the largest amino acid metabolic pool and the tissue with the greatest demand for essential amino acids (EAA) in the animal body, its amino acid composition serves as the optimal reference protein for growing animals [19]. EAA are those that cannot be synthesized by the animal body or cannot meet physiological requirements through synthesis, including leucine, isoleucine, and valine, which are also known as branched-chain amino acids. In this study, threonine and leucine contents were significantly increased in the M15+L30 group, and leucine content was significantly increased in the LCP group. According to Huang et al. [20], branched-chain amino acids promote protein synthesis and inhibit protein degradation, which may explain the increased ADG and carcass CP content observed in the M15+L30 and LCP groups.

For non-essential amino acids (NEAA), aspartic acid, serine, glutamic acid, glycine, alanine, cysteine, and proline contents were significantly increased in the M15+L30 and LCP groups, and tyrosine content was significantly increased in the L30 and M15+L30 groups. Combined with the observed TAA content and the proportions of EAA and NEAA to TAA, these results indicate that supplementation with RPMet 15 g/d + RPLys 30 g/d significantly increased carcass TAA content while altering the compositional ratio of EAA to NEAA. Erasmus et al. [21], Zhen [22], and Wang et al. [23] have demonstrated that rumen microbial amino acid composition is susceptible to variation depending on dietary sources and types, and rumen microbial amino acids represent the primary source of small intestinal amino acids. Therefore, RPMet and RPLys supplementation can effectively supplement limiting amino acids in the small intestine, bringing it closer to the ideal amino acid balance pattern and improving amino acid utilization efficiency, which may account for the significantly increased carcass TAA content observed in this study. Additionally, except for tyrosine, no significant differences were observed in carcass amino acid contents between the LCP and M15+L30 groups, indicating that using RPMet 15 g/d + RPLys 30 g/d to replace 2.20% of dietary CP also positively affects carcass amino acid deposition.

Conclusions

1. Appropriate supplementation with RPMet and RPLys in diets containing 13.40% CP can improve growth performance of Holstein bulls, with combined application at appropriate doses showing better effects.
2. Combined application of RPMet and RPLys at appropriate levels has the potential to replace a portion of dietary CP in Holstein bulls, though its efficacy may vary with dietary type and CP content.

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