Effects of Combined Supplementation of Rumen-Protected Methionine and Cinnamaldehyde on Milk Production Performance and Nitrogen Excretion in Dairy Cows (Postprint)

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Abstract

This study aimed to investigate the effects of combined supplementation of rumen-protected methionine (RPMet) and cinnamaldehyde (CA) on milk production performance and nitrogen excretion in dairy cows. Forty Holstein cows with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90$±$15) d] were selected and allocated into 10 groups with 4 cows per group. The control (C) group was fed a basal diet, while the treatment groups were supplemented with different level combinations of RPMet and CA. RPMet was set at three levels: 20 (L), 25 (M), and 30 (H) g/(d · head); CA was set at three levels: 15 (L), 18 (M), and 21 (H) g/(d · head), forming nine different level combinations: LL, ML, HL, LM, MM, HM, LH, MH, and HH (the first letter indicates RPMet supplementation level, the second letter indicates CA supplementation level). The preliminary period was 15 d, and the formal experimental period was 60 d. The results showed: 1) Except for the LH group, milk yield in all treatment groups was significantly or extremely significantly higher than that in the C group (P<0.05 or P<0.01), with the HL group being the highest. 2) Milk fat percentage and milk protein percentage in all treatment groups were higher than those in the C group, both being highest in the HL group, with extremely significant differences from the C group (P<0.01); somatic cell count (SCC) in all treatment groups was lower than that in the C group, being lowest in the HL group, with extremely significant differences from the C group (P<0.01). 3) Except for the LH group, apparent nitrogen digestibility and nitrogen conversion efficiency for lactation in all treatment groups were significantly or extremely significantly higher than those in the C group (P<0.05 or P<0.01), with the HL group being the highest; total nitrogen excretion in the LL, ML, HL, LM, MM, HM, LH, MH, and HH groups was reduced by 17.45% (P<0.01), 18.79% (P<0.01), 20.80% (P<0.01), 10.41% (P<0.01), 12.49% (P<0.01), 15.22% (P<0.01), 3.37% (P>0.05), 5.12% (P<0.05), and 7.43% (P<0.05) compared with the C group, respectively, with the HL group being the lowest. The results suggest that supplementation of RPMet and CA in the diet of lactating dairy cows can improve milk production performance and reduce nitrogen excretion; considering the above indicators comprehensively, the optimal combination is RPMet 30 g/(d · head) and CA 15 g/(d · head).

Full Text

Effects of Rumen-Protected Methionine and Cinnamic Aldehyde on Lactation Performance and Nitrogen Excretion of Dairy Cows

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Abstract

This experiment was conducted to investigate the effects of combined supplementation of rumen-protected methionine (RPMet) and cinnamic aldehyde (CA) on 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$±$15) days] were allocated to 10 groups with 4 cows per group. The control (C) group received a basal diet, while experimental groups were supplemented with different level combinations of RPMet and CA. RPMet was added at three levels: 20 (L), 25 (M), and 30 (H) g/(d · head), and CA was added at three levels: 15 (L), 18 (M), and 21 (H) g/(d · head), forming nine different combinations: LL, ML, HL, LM, MM, HM, LH, MH, and HH (the first letter indicates RPMet level, the second indicates CA level). The preliminary period lasted 15 days, followed by a 60-day formal experimental period. The results showed that: (1) Except for the LH group, milk yield in all experimental groups was significantly or extremely significantly higher than in the C group (P<0.05 or P<0.01), with the HL group achieving the highest yield. (2) Milk fat percentage and milk protein percentage in all experimental groups exceeded those of the C group, with the HL group showing the highest values and extremely significant differences from the C group (P<0.01). Milk somatic cell counts in all experimental groups were lower than in the C group, with the HL group having the lowest count and extremely significant differences from the C group (P<0.01). (3) Except for the LH group, nitrogen apparent digestibility and nitrogen conversion efficiency of lactation in experimental groups were significantly or extremely significantly higher than in the C group ($P<0.05$ or $P<0.01$), with the HL group showing the highest values. Total nitrogen excretion in the LL, ML, HL, LM, MM, HM, LH, MH, and HH groups decreased by 17.45% ($P<0.01$), 18.79% ($P<0.01$), 20.80% ($P<0.01$), 10.41% ($P<0.01$), 12.49% ($P<0.01$), 15.22% ($P<0.01$), 3.37% ($P>0.05$), 5.12% ($P<0.05$), and 7.43% ($P<0.05$) respectively compared to the C group, with the HL group showing the lowest excretion. These results indicate that dietary supplementation with RPMet and CA can improve lactation performance and reduce nitrogen excretion in dairy cows. Considering all measured indicators, the optimal combination is 30 g/(d · head) RPMet and 15 g/(d · head) CA.

Keywords: rumen-protected methionine; cinnamic aldehyde; lactation performance; nitrogen excretion

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Introduction

The rapid development of China' s dairy industry, particularly the increasing intensification and scale of operations, has effectively alleviated market supply-demand contradictions. However, dairy manure has simultaneously caused severe environmental pollution, with nitrogen pollution recognized as a major environmental factor. The rumen of ruminants represents a relatively stable anaerobic fermentation system characterized by dynamic equilibrium relationships—both synergistic and restrictive—between host and microorganisms as well as among microbial populations. Due to rumen microbial degradation of dietary protein and deamination of amino acids, dietary protein cannot efficiently provide amino acids for rumen microorganisms and the host. Excessive ammonia nitrogen (NH_3-N) exceeds microbial utilization capacity, resulting in wasted dietary protein. Therefore, employing nutritional regulation techniques to improve dietary protein utilization and reduce nitrogen excretion without compromising lactation performance holds significant practical importance for addressing nitrogen pollution in dairy production.

Methionine serves as the first or second limiting amino acid for ruminants, particularly crucial for high-yielding dairy cows and often termed the "life amino acid." Previous research demonstrated that supplementing early-lactation Holstein cows with rumen-protected methionine (RPMet) significantly increased milk yield, milk protein percentage, and milk specific gravity while also elevating milk fat percentage and non-fat solids content. In beef cattle, intraruminal administration of animal fat-coated methionine significantly improved digestible nitrogen and retained nitrogen. Cinnamic aldehyde (CA), also known as cinnamaldehyde or phenylacrolein, is a yellow liquid obtainable through both plant extraction (from cinnamon) and artificial synthesis. Low-level cinnamon oil supplementation has been shown to reduce milk urea nitrogen content and somatic cell counts. In beef cattle, dietary CA supplementation at 300, 600, and 900 mg/(d · head) produced a significant linear increase in feed conversion efficiency.

Our research group previously investigated the individual effects of RPMet and CA on dairy cow lactation performance and nitrogen excretion, establishing optimal supplemental levels of 25 g/(d • head) for RPMet and 18 g/(d • head) for CA. However, few studies have examined the combined effects of RPMet and CA on dairy cow lactation performance and nitrogen excretion, and the optimal combination remains unclear. Building upon these previous optimal levels, this experiment established three RPMet levels (25$±5*g*)*andthreeCAlevels*(18±$3 g), creating nine different combinations to determine the optimal RPMet-CA supplementation strategy. The objective was to improve dietary protein utilization and lactation performance while reducing nitrogen excretion, thereby providing a theoretical basis for combined RPMet and CA application in dairy production.

Materials and Methods

1.1 Experimental Design This study utilized 40 Holstein dairy cows from Qingdao Aote Dairy Farm with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90$±$15) days]. Cows were randomly divided into 10 groups (4 cows per group). The control (C) group received a basal diet, while experimental groups received different RPMet and CA level combinations. RPMet was supplemented at three levels: 20, 25, and 30 g/(d • head), and CA at three levels: 15, 18, and 21 g/(d • head), forming nine combinations as detailed in Table 1 .

Each cow received 0.5 kg of concentrate daily as a carrier mixed with RPMet and CA. The remaining concentrate was blended with forage to formulate total mixed rations (TMR). Supplements were mixed with concentrate and fed with TMR. The total experimental period was 75 days, comprising a 15-day preliminary period and a 60-day formal experimental period. RPMet (rumen bypass rate 85%) and CA were purchased from Qingdao Runbot Biological Technology Co., Ltd. RPMet was a white granular material composed of DL-methionine and silica (DL-methionine $ $60%, moisture $ 12 $5%, moisture $ $12%).

1.2 Feeding Management Cows were milked twice daily (04:00 and 16:00) using a DeLaval milking machine and fed TMR twice daily (04:30 and 16:30), ensuring access to TMR for over 20 hours per day. Post-feeding, cows had ad libitum access to water and exercise in a paddock. Routine deworming, lighting, and management protocols were followed. TMR composition and nutrient levels are presented in Table 2 .

1.3 Sample Collection and Analysis 1.3.1 Feed Intake Measurement

Cows were fed in individual stalls with feed intake recorded separately. During the preliminary period, feed offered and refused was recorded on days 1-3, 5-7, 9-11, and 13-15 to calculate individual intake. Following the preliminary period, average intake was calculated. During the formal period, intake was recorded every 10 days (six total recordings), with three consecutive days recorded each time. Average intake was used to adjust TMR allocation for the subsequent period. Final average intake across all six recordings was calculated for nutrient intake determination.

1.3.2 TMR and Fecal Sample Collection and Analysis

TMR samples were collected using the quartering method, oven-dried at 65°C, and ground for analysis. Fecal samples were collected three times: days 1-3 of the preliminary period, days 28-30 of the formal period, and days 58-60 of the formal period. Total fecal collection was employed, with samples obtained from all four cows per group. Before collection, stalls were thoroughly cleaned. Daily feces were mixed, weighed, and quartered for sample collection. Sulfuric acid (25 mL of 10% solution per 100 g feces) was added for nitrogen fixation before storage at -20°C. After sampling, three-day samples were proportionally mixed, oven-dried at 65°C to constant weight, and stored.

TMR samples were analyzed for: moisture content (GB/T6435-2006) to calculate dry matter (DM); crude protein (CP) by Kjeldahl method (GB/T 6432-1994); acid detergent fiber (ADF) by NY/T 1459-2007; neutral detergent fiber (NDF) by GB/T20806-2006; calcium (Ca) by permanganate method (GB/T6436-2002); and phosphorus (P) by spectrophotometry (GB/T6437-2002). Fecal CP analysis followed the same method as TMR.

1.3.3 Urine Sample Collection and Analysis

Urine samples were collected three times: days 1-3 of the preliminary period, days 28-30 of the formal period, and days 58-60 of the formal period. Sampling followed the spot urine collection method, combining manual collection with bladder catheterization. Cows were restrained using neck clamps, and catheters were inserted to collect urine. If spontaneous urination occurred during collection, personnel manually collected the sample. Urine was collected twice daily at 12-hour intervals for three consecutive days, with collection times delayed by 4 hours each day. Concentrated sulfuric acid (98%) was added to adjust pH (<3), and samples were stored at -20°C.

1.3.4 Milk Sample Collection and Analysis

Milk yield was recorded automatically during twice-daily milking (04:00, 16:00) using a DeLaval herringbone milking system. Milk yield was recorded every 5 days during both preliminary and formal periods, with three consecutive days recorded each time and values averaged.

On days 15, 30, 45, and 60 of the formal period, 65 mL of milk samples were collected proportionally to morning and evening yields. Fifty milliliters were preserved with potassium dichromate (0.6 mg/mL), mixed, and stored at 4°C for milk composition analysis. The remaining 15 mL was centrifuged to remove fat and protein; 1.5 mL of the processed sample was stored at -20°C for milk urea nitrogen determination. Milk fat percentage, protein percentage, lactose percentage, and somatic cell count were analyzed using a CombiFoss FT+ automatic analyzer (Foss, Denmark) at the Dairy Performance Testing Laboratory of Shandong Academy of Agricultural Sciences. Formal period composition was calculated using weighted averages.

1.3.5 Nitrogen Metabolism Indices

Urinary nitrogen was determined using a Foss KjeltecTM 8200 Kjeldahl analyzer (Foss, Denmark). Urinary creatinine was measured by picric acid colorimetry using a UV-1800PC spectrophotometer (Shanghai Mapada Instruments Co., Ltd.) with assay kits from Nanjing Jiancheng Bioengineering Institute. Daily urine volume was estimated using urinary creatinine as a marker (approximately 29 mg creatinine excreted per kg body weight daily) following Valadares et al.

Nitrogen metabolism calculations: - Fecal nitrogen (g/d) = daily fecal output × fecal crude protein content × 0.16 - Total nitrogen excretion (g/d) = fecal nitrogen + urinary nitrogen - Nitrogen apparent digestibility (%) = [(nitrogen intake - fecal nitrogen) / nitrogen intake] × 100 - Nitrogen conversion efficiency of lactation (%) = (milk nitrogen / nitrogen intake) × 100

1.4 Data Processing and Analysis Data were initially processed using Excel 2016. One-way ANOVA was performed using SPSS 20.0 software, with Duncan' s multiple comparison test used to assess inter-group differences. Significance was declared at $P<0.05$ and extreme significance at $P<0.01$. Results are expressed as means ± standard error.

Results

2.1 Effects of Different RPMet and CA Combinations on Nutrient Intake As shown in Table 3 , dietary supplementation with different RPMet and CA combinations tended to increase main nutrient intake in dairy cows, though no significant differences were observed between experimental and C groups ($P>0.05$).

2.2 Effects of Different RPMet and CA Combinations on Milk Yield Table 4 demonstrates that milk yield in the LL, ML, HL, LM, MM, HM, LH, MH, and HH groups increased by 16.07% ($P<0.01$), 19.40% ($P<0.01$), 22.17% ($P<0.01$), 12.49% ($P<0.01$), 13.46% ($P<0.01$), 15.27% ($P<0.01$), 3.94% ($P>0.05$), 6.60% ($P<0.05$), and 9.30% ($P<0.01$) respectively compared to the C group, with the HL group achieving the highest yield.

2.3 Effects of Different RPMet and CA Combinations on Milk Composition Table 5 reveals that milk fat percentage was highest in the HL group, which was extremely significantly higher than the C, LM, LH, MH, and HH groups (P<0.01) and significantly higher than the MM group (P<0.05). Milk protein percentage was also highest in the HL group, showing extremely significant differences from the C, LM, LH, MH, and HH groups (P<0.01) and significant differences from the MM and HM groups (P<0.05). Milk somatic cell count decreased following RPMet and CA supplementation, with the HL group exhibiting the lowest count and an extremely significant difference from the C group (P<0.01). No significant differences in lactose percentage were observed among groups (P>0.05).

2.4 Effects of Different RPMet and CA Combinations on Nitrogen Apparent Digestibility and Excretion As presented in Table 6 , RPMet and CA supplementation reduced both fecal and urinary nitrogen excretion. Total nitrogen excretion in the LL, ML, HL, LM, MM, HM, LH, MH, and HH groups decreased by 17.45% (P<0.01), 18.79% (P<0.01), 20.80% (P<0.01), 10.41% (P<0.01), 12.49% (P<0.01), 15.22% (P<0.01), 3.37% (P>0.05), 5.12% (P<0.05), and 7.43% (P<0.05) respectively compared to the C group, with the HL group showing the lowest excretion. Nitrogen apparent digestibility and nitrogen conversion efficiency of lactation increased in all experimental groups, with values significantly or extremely significantly higher than the C group (except for the LH group) (P<0.05 or P<0.01).

Discussion

3.1 Effects of RPMet and CA Combinations on Nutrient Intake Dry matter intake (DMI) critically influences dairy cow performance, and increased DMI provides more nutrients and energy for lactation. In this experiment, groups receiving lower CA levels exhibited relatively higher DMI, suggesting that low-level CA tended to increase DMI, while RPMet had minimal effect on DMI. Previous studies reported that N-acetyl-DL-methionine tended to increase DMI in sheep, and a garlic oil-CA complex also improved DMI in dairy cows. The enhanced DMI observed with RPMet and CA supplementation may be attributed to improved rumen environment and enhanced nutrient digestion and absorption. Cinnamic aldehyde can increase saliva secretion, and the alkaline nature of saliva helps maintain stable rumen pH. Additionally, small amounts of methionine released from RPMet in the rumen can improve the nutritional environment, promoting microbial growth and reproduction and accelerating feed degradation. Furthermore, CA promotes digestive juice secretion, enhances digestive function, and relieves gastrointestinal smooth muscle spasms, all contributing to increased DMI.

3.2 Effects of RPMet and CA Combinations on Milk Yield Milk yield represents a key indicator of lactation performance. Under our experimental conditions, groups receiving lower CA levels showed significantly improved milk yield. When CA supplementation level was constant, higher RPMet levels produced greater milk yield increases, with the HL group performing best. Previous studies demonstrated that RPMet and garlic oil-CA complexes both enhanced milk yield in dairy cows. The lactation system is primarily regulated by the growth hormone (GH) axis, with GH treatment increasing milk yield by 36% in early-lactation cows. GH and insulin (INS) interact to accelerate mammary metabolism and promote nutrient transport to the mammary gland, providing more precursors for milk synthesis. Dietary RPMet and CA supplementation can increase serum GH and insulin-like growth factor I (IGF-I) concentrations. IGF-I promotes mammary gland development and mammary cell proliferation, indirectly stimulating lactation. Cinnamic aldehyde reduces dietary protein degradation in the rumen, increasing amino acid flow to the small intestine and positively affecting milk yield. Moreover, both RPMet and CA improve rumen environment, promote microbial growth, and enhance nutrient digestion and absorption, further supporting increased milk production.

3.3 Effects of RPMet and CA Combinations on Milk Composition
Milk fat and protein percentages are important indicators of milk quality. In this experiment, RPMet and CA supplementation improved both parameters. Previous research showed that 12 g/d RPMet supplementation during summer increased milk fat and protein percentages while reducing somatic cell counts. A garlic oil-CA complex significantly increased milk yield and decreased somatic cell counts in early-lactation cows. During peak lactation, milk protein percentage is typically low because DMI increase lags behind milk yield increase, creating negative energy balance. Therefore, RPMet supplementation during early lactation is essential for maintaining milk quality. Direct methionine supplementation is largely degraded by rumen microorganisms, limiting intestinal absorption and negating its benefits. Our results indicate that when CA level was constant, higher RPMet levels more effectively increased milk fat and protein percentages, with the HL group showing optimal results. The improvement may be explained by increased GH and INS concentrations. GH promotes synthesis of acetyl-CoA carboxylase, fatty acid synthase, and lipoprotein lipase (with acetyl-CoA carboxylase being the rate-limiting enzyme in fatty acid synthesis), enhancing fatty acid production and providing more precursors and energy for lactation. INS is essential for mammary development and function, directly regulating mammary protein synthesis. The interaction between GH and INS significantly increases milk yield and protein production in early-lactation cows. Additionally, RPMet and CA improve rumen nutritional environment, promoting microbial protein synthesis and providing more precursors for milk protein synthesis. The small amount of rumen-protected fat in RPMet provides substrates for milk fat synthesis, while CA' s hypoglycemic and lipid-regulating effects also contribute to increased milk fat percentage.

Milk somatic cell count serves as a crucial indicator of milk quality and udder health, with lower counts indicating better udder health and lower subclinical mastitis incidence. Simultaneous RPMet and CA supplementation reduced somatic cell counts, suggesting improved udder health. Both supplements enhance immunity: RPMet scavenges free radicals, reduces lymphocyte apoptosis, and improves immunity and antioxidant capacity, while CA significantly increases lymphocyte proliferation and macrophage phagocytic activity. Additionally, CA exhibits strong antibacterial properties; its aldehyde group, being hydrophilic, readily adsorbs to fungal surface hydrophilic groups, disrupting cell wall polysaccharide structures and penetrating cell walls. These antimicrobial and immunomodulatory functions collectively reduce somatic cell counts and improve udder health.

3.4 Effects of RPMet and CA Combinations on Nitrogen Apparent Digestibility and Excretion Ammonia nitrogen loss in the rumen represents a major factor limiting dietary protein utilization in dairy cows. Therefore, nutritional strategies to improve NH_3-N utilization are crucial for enhancing nitrogen efficiency and reducing excretion. Methionine, as the first or second limiting amino acid, not only affects amino acid balance but also limits utilization of other amino acids when deficient. Unutilized amino acids are converted to urea via the ornithine cycle and excreted in urine. Our results show that groups receiving lower CA levels exhibited extremely significant reductions in total nitrogen excretion. When CA level was constant, higher RPMet levels produced greater reductions in nitrogen excretion, with the HL group performing optimally. Cinnamic aldehyde reduces rumen protein degradation, decreasing nitrogen loss from rapid protein breakdown. Small amounts of methionine released from RPMet improve rumen nutritional environment, promoting microbial growth and accelerating NH_3-N utilization, thereby reducing rumen NH_3-N loss. The regulatory effects of RPMet and CA on NH_3-N production and utilization rates improve their balance, enhance nitrogen efficiency, and reduce excretion. Effective methionine release from RPMet in the post-rumen digestive tract increases intestinal methionine supply, balances amino acid composition, improves intestinal amino acid utilization, and reduces nitrogen excretion. Cinnamic aldehyde promotes butyrate secretion in the intestine, which stimulates intestinal cell proliferation and pancreatic enzyme secretion, enhancing nutrient absorption and further reducing nitrogen excretion.

In conclusion, combined dietary supplementation of RPMet and CA improves lactation performance and reduces nitrogen excretion in dairy cows. Based on comprehensive evaluation of all measured indicators, the optimal combination is 30 g/(d · head) RPMet and 15 g/(d · head) CA.

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