Effects of Cinnamaldehyde on Urinary Purine Derivative Excretion, Milk Production Performance, and Nitrogen Excretion in Dairy Cows (Postprint)

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Abstract

This experiment aimed to investigate the effects of cinnamaldehyde (CA) on urinary purine derivative excretion, milk production 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$±$15) d] were selected and randomly divided into 4 groups with 10 cows per group. The control group and experimental groups 1, 2, and 3 were supplemented with 0, 12, 18, and 24 g/(d·head) cinnamaldehyde in the diet, respectively. The preliminary period was 15 d, and the formal experimental period was 60 d. The results showed: 1) Urinary purine derivative excretion in all experimental groups was extremely significantly higher than that in the control group ($P<0.01$), with increases of 14.22%, 17.62%, and 10.49% in experimental groups 1, 2, and 3 compared with the control group, respectively. 2) Milk yield in all experimental groups was significantly or extremely significantly higher than that in the control group ($P<0.05$ or $P<0.01$), with increases of 10.80%, 12.15%, and 6.48% in experimental groups 1, 2, and 3 compared with the control group, respectively; milk fat percentage in experimental groups 1 and 2 was extremely significantly higher than that in the control group ($P<0.01$); milk protein percentage in all experimental groups was extremely significantly higher than that in the control group ($P<0.01$); milk somatic cell count in all experimental groups was extremely significantly lower than that in the control group ($P<0.01$). 3) Total nitrogen excretion in all experimental groups was extremely significantly lower than that in the control group ($P<0.01$), with decreases of 9.76%, 14.13%, and 7.39% in experimental groups 1, 2, and 3 compared with the control group, respectively. It can be concluded that under the conditions of this experiment, considering the comprehensive indicators of urinary purine derivative excretion, milk yield, milk composition, and total nitrogen excretion, the optimal supplementation

level of cinnamaldehyde was 18 g/(d · head).

Full Text

Effects of Cinnamic Aldehyde on Urinary Purine Derivatives Excretion, Milk Performance and Nitrogen Excretion in Dairy Cows

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Abstract

This experiment was conducted to investigate the effects of cinnamic aldehyde (CA) on urinary purine derivatives excretion, milk performance, and nitrogen excretion in dairy cows. Forty Holstein cows with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90$±$15) days in milk] were randomly allocated into four groups with ten cows per group. The control group and test groups 1, 2, and 3 were supplemented with 0, 12, 18, and 24 g/(d · head) of cinnamic aldehyde in their diets, respectively. The pre-experimental period lasted for 15 days, followed by a 60-day formal experimental period. The results showed that: (1) Urinary purine derivatives excretion in all test groups was significantly higher than that in the control group ($P<0.01$), with increases of 14.22%, 17.62%, and 10.49% observed in test groups 1, 2, and 3, respectively. (2) Milk yield in all test groups was significantly or highly significantly higher than that in the control group ($P<0.05$ or $P<0.01$), with test groups 1, 2, and 3 showing increases of 10.80%, 12.15%, and 6.48%, respectively. Milk fat percentage in test groups 1 and 2 was highly significantly higher than that in the control group ($P<0.01$). Milk protein percentage in all test groups was highly significantly higher than that in the control group ($P<0.01$), while milk somatic cell count in all test groups was highly significantly lower than that in the control group ($P<0.01$). (3) Total nitrogen excretion in all test groups was highly significantly lower than that in the control group ($P<0.01$), with reductions of 9.76%, 14.13%, and 7.39% observed in test groups 1, 2, and 3, respectively. Based on these results, it can be concluded that under the conditions of this experiment, the optimal supplementation level of cinnamic aldehyde is 18 g/(d·head) when considering urinary purine derivatives excretion, milk yield, milk composition, and total nitrogen excretion.

Key words: cinnamic aldehyde; purine derivatives; milk performance; nitrogen excretion

Introduction

In recent years, with the rapid development of China' s dairy industry, domestic protein feed resources have become increasingly scarce, with protein sources such as soybeans relying heavily on imports. Consequently, improving protein utilization efficiency through nutritional regulation techniques has remained a hot topic in animal nutrition research. Meanwhile, intensive and large-scale dairy farming models have resulted in substantial amounts of unused nitrogen being discharged into the environment, causing environmental pollution and rising feeding costs that have become important factors restricting the sustainable development of China' s dairy industry. Improving protein utilization efficiency and reducing nitrogen excretion through nutritional regulation techniques without affecting dairy cow performance holds positive significance for the development of China' s dairy industry.

Cinnamic aldehyde (CA), also known as cinnamaldehyde, cinnamal, or 3-phenyl-2-propenal, is a yellow liquid that can be extracted from plants such as cinnamon or obtained through artificial synthesis [1]. Zhang et al. [2] reported that supplementing dairy cow diets with a garlic oil and cinnamic aldehyde complex (GAR-CIN) significantly increased milk yield, significantly reduced milk somatic cell count, and improved nutrient digestibility. Research has demonstrated that adding yucca and cinnamon plant extracts to broiler diets can improve feed nitrogen utilization efficiency and reduce excretion of urea nitrogen, ammonia nitrogen (NH_3-N), and total nitrogen, thereby decreasing environmental pollution [3]. Currently, research on cinnamic aldehyde in ruminant production has primarily focused on its effects on rumen microbial fermentation and methane production, while few studies have reported on the effects of dietary cinnamic aldehyde supplementation on urinary purine derivatives (PD) excretion, milk performance, and nitrogen excretion. Therefore, this experiment was designed to investigate the effects of different levels of cinnamic aldehyde supplementation on urinary purine derivatives excretion, milk performance, and nitrogen excretion in dairy cows, aiming to determine the optimal supplementation level of cinnamic aldehyde in dairy cow diets to improve protein feed utilization efficiency, enhance milk performance, reduce feeding costs and nitrogen excretion, and provide technical support for the sustainable development of China' s dairy industry.

1.1 Experimental Design

This experiment employed a single-factor randomized block design. Forty Holstein cows with similar age, body weight, parity, milk yield, milk composition, and lactation stage [(90$±$15) days in milk] were selected from Qingdao Aote Dairy Farm and randomly divided into four groups with ten cows per group.

The control group and test groups 1, 2, and 3 were supplemented with 0, 12, 18, and 24 g/(d • head) of cinnamic aldehyde in their diets, respectively. Each cow was reserved 0.5 kg of concentrate daily as a carrier to be mixed with cinnamic aldehyde. The remaining concentrate was mixed with roughage to form a total mixed ration (TMR). The composition and nutrient levels of the TMR are presented in Table 1 . Cinnamic aldehyde was mixed with concentrate and fed with the TMR. The entire experimental period lasted for 75 days, including a 15-day pre-experimental period and a 60-day formal experimental period. The cinnamic aldehyde used in this experiment was a cinnamic aldehyde complex provided by Qingdao Runbot Biological Technology Co., Ltd., appearing as a white powder containing cinnamic aldehyde, silicon dioxide, and starch, with cinnamic aldehyde content $ $5% and moisture $ $12%.

1.2 Feeding Management

Cows were fed in separate pens with individual feed intake recorded. During the pre-experimental period, TMR refusal was weighed every two days for a total of six recordings, with feed delivery amounts also recorded. Refusals from the previous feeding were collected and weighed before each new feeding, and individual cow feed intake was calculated based on delivery and refusal amounts. After the pre-experimental period, average feed intake during this period was calculated from the six recordings. During the formal experimental period, feed intake was recorded every ten days for a total of six recordings, with each recording lasting three consecutive days. Average feed intake was calculated from each three-day recording and used to adjust TMR delivery amounts for the subsequent period. After the formal experimental period, average feed intake during this period was calculated from the six recordings. Cows were milked twice daily (04:00 and 16:00) using Lely milking machines and fed TMR twice daily (04:30 and 16:30), ensuring cows had access to TMR for more than 20 hours per day. After feeding, cows had free access to water and exercise in the exercise yard, and were managed according to routine practices for deworming, lighting, and other management procedures.

1.3 Sample Collection and Analysis

1.3.1 Urine Samples Urine samples were collected three times: on days 1-3 of the pre-experimental period, days 28-30 of the formal experimental period, and days 58-60 of the formal experimental period. Sampling was conducted using the spot urine collection method described by Zhu [6], with manual collection combined with bladder catheterization. Urine was collected twice daily at 12-hour intervals for three consecutive days, with collection times delayed by 4 hours each day compared to the previous day. Collected urine was acidified with 98% concentrated sulfuric acid to adjust pH (pH<3) and stored at -20°C. Uri-

nary nitrogen content was analyzed using the Kjeldahl method [5], urea nitrogen content was determined using the urease method [7], and creatinine content was measured using the picric acid colorimetric method [8]. All assay kits were purchased from Nanjing Jiancheng Bioengineering Institute. Following the method of Valadares et al. [8], urinary creatinine (approximately 29 mg excreted per kg body weight per day) was used as a marker to estimate daily urine volume.

1.3.2 Feed and Fecal Samples TMR samples were collected using the quartering method, dried at 65°C to produce air-dried samples, and ground for subsequent analysis. Fecal samples were collected three times using the total fecal collection method on days 1–3 of the pre-experimental period, days 28–30 of the formal experimental period, and days 58–60 of the formal experimental period. Feces were collected continuously for 24 hours over three days from all ten cows in each group. Before collection, stalls were thoroughly cleaned, and feces were collected promptly, mixed, and weighed daily. Daily fecal samples were collected using the quartering method, treated with 25 mL of 10% sulfuric acid per 100 g of feces for nitrogen fixation, and stored at -20°C. On the final day of each sampling period, the three-day fecal samples were mixed uniformly according to weight proportions, then dried to constant weight at 65°C. Crude protein (CP) content in diets and feces was determined using methods described in "Feed Analysis and Feed Quality Detection Technology" edited by Zhang [5].

1.3.3 Milk Yield and Composition Cows were milked twice daily (04:00 and 16:00) using Lely herringbone milking machines with automatic milk yield recording. Milk yield was recorded every five days during both the pre-experimental and formal experimental periods, with each recording lasting three consecutive days and average values calculated.

Milk samples were collected on day 1 of the pre-experimental period and every 15 days during the formal experimental period, with samples proportionally collected based on morning and evening milk yields. A total of 65 mL was collected, with 50 mL preserved with potassium dichromate (0.6 mg/mL) and stored at 4°C for milk composition analysis. The remaining 15 mL was centrifuged to remove milk fat and protein, and 1.5 mL of the processed sample was stored at -20°C for milk urea nitrogen determination. Milk fat percentage, milk protein percentage, lactose percentage, and somatic cell count were measured using a milk composition and somatic cell automatic analyzer (CombiFoss FT+, Foss, Denmark) at the Dairy Performance Testing Laboratory of Shandong Academy of Agricultural Sciences. Weighted averages were used to calculate milk composition content during the formal experimental period.

1.3.4 Urinary Purine Derivatives Excretion Urinary purine derivatives primarily originate from rumen microbial purines, allowing rumen microbial protein (MCP) production to be estimated through purine derivatives measurement. Urinary uric acid and allantoin contents were determined using uric

acid assay kits and enzyme-linked immunosorbent assay (ELISA) kits, respectively. For uric acid determination, uric acid in protein-free filtrate reduces phosphotungstic acid to tungsten blue, allantoin, and carbon dioxide under alkaline conditions, with blue color intensity proportional to uric acid content. Colorimetric measurement was performed using a UV-1800PC spectrophotometer (Shanghai Mapada Instruments Co., Ltd.) to calculate uric acid content. For allantoin determination, samples, standards, and horseradish peroxidase (HRP)-labeled detection antibodies were added sequentially to microplates precoated with allantoin antibodies. After incubation and thorough washing, the substrate 3,3' ,5,5' -tetramethylbenzidine (TMB) was added for color development. TMB is converted to blue under catalase action and finally to yellow under acidic conditions, with color intensity positively correlated with allantoin content. Absorbance was measured using an MK3 microplate reader (Thermo Fisher Scientific, Shanghai) and allantoin content was calculated from the standard curve [9]. Total urinary purine derivatives content was calculated as the sum of uric acid and allantoin [10] using the following formula:

Daily total urinary purine derivatives excretion (mmol/d) = daily uric acid excretion (mmol/d) + daily allantoin excretion (mmol/d)

1.4 Data Processing and Analysis

Experimental data were initially processed using Excel 2016 software. One-way ANOVA was performed using SPSS 20.0 software, and Duncan' s multiple comparison test was used to examine significant differences among groups. Differences were considered significant at $P<0.05$ and highly significant at $P<0.01$. Results are expressed as means $\pm$ standard error.

2.1 Effects of Cinnamic Aldehyde on Urinary Purine Derivatives Excretion in Dairy Cows

As shown in Table 2 , uric acid content in test groups 1 and 2 was highly significantly higher than that in the control group ($P<0.01$), while test group 3 was significantly higher than the control group ($P<0.05$), with no significant differences among test groups ($P>0.05$). For allantoin content, all test groups were highly significantly higher than the control group ($P<0.01$), with test group 2 being highly significantly higher than test group 3 ($P<0.01$). No significant differences were observed between test groups 2 and 1 or between test groups 3 and 1 ($P>0.05$). Regarding purine derivatives excretion, all test groups were highly significantly higher than the control group ($P<0.01$), with test group 2 being highly significantly higher than test group 3 ($P<0.01$). No significant differences were found between test groups 2 and 1 or between test groups 3 and 1 ($P>0.05$). Compared with the control group, purine derivatives excretion in test groups 1, 2, and 3 increased by 14.22%, 17.62%, and 10.49%, respectively.

Table 2 Effects of cinnamic aldehyde on urinary purine derivatives excretion in dairy cows

Items	Control group	Test group 1	Test group 2	Test group 3
Uric acid/(mmol/d)	32.13$±0.79 < sup > Bb < /sup > |42.63±1.99 < sup > Aa < /sup > |45.93±2.88 < sup > Aa < /sup > |40.75±1.01 < sup > ABa < /sup > ||Allantoin/(mmol/d)|271.50±3.40 < sup > Cc < /sup > |304.20±3.30 < sup > ABab < /sup > |311.23±2.87 < sup > Aa < /sup > |294.73±1.94 < sup > Bb < /sup > ||PD/(mmol/d)|303.65±3.69 < sup > Cc < /sup > |346.83±3.53 < sup > ABab < /sup > |357.15±2.97 < sup > Aa < /sup > |335.50±$4.18Bb			
Increase range of PD/%	-	14.22	17.62	10.49

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), different capital letter superscripts indicate highly

significant difference (P<0.01), and same or no letter superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects of Cinnamic Aldehyde on Dry Matter Intake and Milk Performance in Dairy Cows

As shown in Table 3 , cinnamic aldehyde had minimal effects on dry matter intake (DMI), with no significant differences between test groups and the control group (P>0.05). For milk yield, test groups 1 and 2 were highly significantly higher than the control group (P<0.01), while test group 3 was significantly higher than the control group (P<0.05). No significant differences were observed between test groups 2 and 1, between test groups 3 and 1, or between test groups 3 and 2 (P>0.05). Milk yield in test groups 1, 2, and 3 increased by 10.80%, 12.15%, and 6.48% compared with the control group, respectively. For milk fat percentage, test groups 1 and 2 were highly significantly higher than the control group (P<0.01), with test group 2 being significantly higher than test group 1 (P<0.05) and highly significantly higher than test group 3 (P<0.01). No significant difference was found between test group 3 and the control group (P>0.05). Milk protein percentage in all test groups was highly significantly higher than that in the control group (P<0.01), with test group 2 being highly significantly higher than test groups 1 and 3 (P<0.01), while no significant difference existed between test groups 1 and 3 (P>0.05). For milk somatic cell count, all test groups were highly significantly lower than the control group (P<0.01), with test group 2 being highly significantly lower than test group 3 (P<0.01). No significant differences were observed between test groups 2 and 1 or between test groups 3 and 1 (P>0.05).

Table 3 Effects of cinnamic aldehyde on dry matter intake and milk performance in dairy cows

Items	Control group	Test group 1	Test group 2	Test group 3
Items	Control group	Test group 1	Test group 2	Test group 3
DMI/(kg/d)	21.28$±0.24	21.48±0.16	21.52±0.14	21.39±0.15
$\|Milk yield/(kg/d)$	28.24±0.53 ^{Bc}	31.29±0.48 ^{Aab}	31.67±0.32 ^{Aa}	30.07±0.44 ^{ABb}
$\|Increase range of milk yield/$	±0.05 ^{Cc}	3.61±0.04 ^{ABb}	3.73±0.02 ^{Aa}	3.53±0.02 ^{BCbc}
$\|Milk protein percentage/$	±0.03 ^{Cc}	3.46±0.04 ^{Bb}	3.62±0.02 ^{Aa}	3.43±0.02 ^{Bb}
$\|Milk lactose percentage/$	±0.05	5.20±0.06	5.25±0.09	5.28±0.09
$\|Milk somatic cell counts/(10^{3}/m$	^{Aa}	132.57±4.03 ^{BCbc}	125.97±1.85 ^{Cc}	139.68±$2.95Bb

2.3 Effects of Cinnamic Aldehyde on Nitrogen Excretion in Dairy Cows

As shown in Table 4 , for fecal nitrogen excretion, test groups 1 and 2 were highly significantly lower than the control group ($P<0.01$), with test group 2 being significantly lower than test group 3 ($P<0.05$) but not significantly different from test group 1 ($P>0.05$). Test group 3 was significantly lower than the control group ($P<0.05$) but not significantly different from test group 1 ($P>0.05$). For urinary nitrogen excretion, all test groups were highly significantly lower than the control group ($P<0.01$), with test group 2 being highly significantly lower than test group 3 ($P<0.01$) but not significantly different from test group 1 ($P>0.05$). No significant difference was observed between test groups 3 and 1 ($P>0.05$).

For milk urea nitrogen content, test group 2 was highly significantly lower than the control group ($P<0.01$) and significantly lower than test group 3 ($P<0.05$), but not significantly different from test group 1 ($P>0.05$). Test group 1 was significantly lower than the control group ($P<0.05$), while no significant difference existed between test group 3 and the control group ($P>0.05$). For total nitrogen excretion, all test groups were highly significantly lower than the control group ($P<0.01$), with reductions of 9.76%, 14.13%, and 7.39% observed in test groups 1, 2, and 3, respectively. Test group 2 was significantly lower than test group 1 ($P<0.05$) and highly significantly lower than test group 3 ($P<0.01$), while no significant difference was found between test groups 1 and 3 ($P>0.05$).

For digestible nitrogen content, test group 2 was highly significantly higher than the control group ($P<0.01$) and significantly higher than test group 3 ($P<0.05$), but not significantly different from test group 1 ($P>0.05$). Test groups 1 and 3 were significantly higher than the control group ($P<0.05$), with no significant difference between these two groups ($P>0.05$). For nitrogen apparent digestibility, test groups 1 and 2 were highly significantly higher than the control group ($P<0.01$), with test group 2 being significantly higher than test group 3 ($P<0.05$) but not significantly different from test group 1 ($P>0.05$). Test group 3 was significantly higher than the control group ($P<0.05$) but not significantly different from test group 1 ($P>0.05$).

Table 4 Effects of cinnamic aldehyde on nitrogen excretion in dairy cows

Items	Control group	Test group 1	Test group 2	Test group 3
Items	Control group	Test group 1	Test group 2	Test group 3
Intake N/(g/d)	534.21$±3.52\|537.99±6.01\|539.94±3.90\|537.28±4.07\|\|$FecesN/(g/d)$|179.73±2.27 < $sup > Aa <$ /$sup >$ |166.08±3.96 < $sup > Bbc <$ /$sup >$ |160.41±2.46 < $sup > Bc <$ /$sup >$ |170.22±2.25 < $sup > ABb <$ /$sup >$ ||$UrineN/(g/d)$|219.13±3.14 < $sup > Aa <$ /$sup >$ |193.87±4.36 < $sup > BCbc <$ /$sup >$ |182.09±3.23 < $sup > Cc <$ /$sup >$ |199.17±1.53 < $sup > Bb <$ /$sup >$ ||$MilkN/(g/d)$|147.62±1.60 < $sup > Bc <$ /$sup >$ |173.19±6.28 < $sup > Aab <$ /$sup >$ |183.62±4.80 < $sup > Aa <$ /$sup >$ |165.00±5.32 < $sup > ABb <$ /$sup >$ ||$MUN/(mg/dL)$|16.79±0.40 < $sup > Aa <$ /$sup >$ |14.72±0.51 < $sup > ABbc <$ /$sup >$ |14.24±0.41 < $sup > Bc <$ /$sup >$ |15.89±0.44 < $sup > ABab <$ /$sup >$ ||$DigestibleN/(g/d)$|354.49±1.29 < $sup > Bc <$ /$sup >$ |371.91±3.98 <			

3.1 Effects of Cinnamic Aldehyde on Urinary Purine Derivatives Excretion in Dairy Cows

Jin [11] reported in an in vitro fermentation experiment that adding 500 and 1,500 mg/L of cinnamon oil to dairy cow rumen fermentation fluid significantly increased MCP content after 72 hours of fermentation. Urinary purine derivatives excretion is highly correlated with MCP content, and its excretion level reflects MCP production. Research has demonstrated that the urinary purine derivatives method can accurately estimate MCP production changes and offers advantages such as operational convenience and non-invasiveness [12]. The results of this experiment showed that dietary cinnamic aldehyde supplementation significantly increased urinary purine derivatives excretion, consistent with the above conclusions. Rumen fluid NH_3-N concentration serves as an important indicator for measuring rumen nitrogen metabolism, indirectly reflecting the balance between rumen microbial utilization of NH_3-N for MCP synthesis and rumen microbial degradation of dietary protein to produce NH_3-N. If NH_3-N concentration increases, it indicates that the rate of rumen microbial degradation of dietary protein to produce NH_3-N exceeds the rate of microbial utilization of NH_3-N for MCP synthesis. Conversely, if NH_3-N concentration decreases, it suggests that the rate of rumen microbial utilization of NH_3-N for MCP synthesis exceeds the rate of NH_3-N production [13]. Fraser et al. [14] reported in an in vitro fermentation experiment that adding 500 mg/L of cinnamic aldehyde to rumen fluid significantly reduced NH_3-N concentration. Cardozo et al. [15] found in an in vitro study that cinnamic aldehyde could reduce rumen fluid NH_3-N concentration by inhibiting rumen microbial deamination.

3.2 Effects of Cinnamic Aldehyde on Dry Matter Intake and Milk Performance in Dairy Cows

Cao [16] found that supplementing beef cattle diets with 300, 600, and 900 mg of cinnamic aldehyde daily decreased dry matter intake but linearly increased feed conversion efficiency, while supplementation at 1,200 mg/day highly significantly reduced dry matter intake and decreased feed conversion efficiency. Yang et al. [17-18] reported that dietary supplementation with different levels of cinnamic aldehyde could help improve beef cattle dry matter intake and reduce stress, though the differences were not significant. The results of this experiment indicated that supplementing dairy cow diets with different levels of cinnamic aldehyde had minimal effects on dry matter intake. Milk yield, milk fat percentage, milk protein percentage, and milk somatic cell count are important indicators for evaluating dairy cow performance. Zhang et al. [2] found that supplementing dairy cow diets with 30 g/(d · head) of a garlic oil and cinnamic aldehyde complex significantly increased milk yield by 22.4% and reduced milk somatic cell count by 11.0% compared with the control group,

while having no significant effect on milk fat and protein percentages but showing a trend toward reduced milk urea nitrogen content. Zhou et al. [19] reported that cinnamic aldehyde has hypoglycemic and lipid-regulating effects and can promote glucose conversion to fat. This experiment demonstrated that cinnamic aldehyde supplementation in dairy cow diets increased milk yield, milk fat percentage, and milk protein percentage while reducing milk somatic cell count. Xu et al. [20] investigated the effects of NE300, a plant extract based on garlic and cinnamon, on performance of early-lactation dairy cows and found that NE300 could reduce protein degradation in the rumen and significantly increase milk yield while highly significantly reducing milk somatic cell count. Kung et al. [21] reported that reducing protein degradation rate in the rumen to increase amino acid flow to the small intestine is a common practice for improving dairy cow milk yield. Taylor et al. [22] found that increasing dietary protein bypass rate could improve dairy cow milk yield, milk fat percentage, and lactose percentage. Cardozo et al. [15] reported that low-dose cinnamon oil could reduce milk urea nitrogen and somatic cell count, possibly due to its effects on rumen microbial nitrogen metabolism, which increased amino acid content and reduced ammonia production. Milk somatic cell count is an important indicator for evaluating udder health; lower somatic cell counts indicate better udder health and lower incidence of subclinical mastitis. Sung et al. [23] studied the effects of cinnamic aldehyde on 3-week-old broilers and found that cinnamic aldehyde could improve immune function, with 25–400 ng/mL highly significantly enhancing splenic lymphocyte proliferation, 1.2–5.0 g/mL highly significantly activating macrophage phagocytosis, and 14.4 mg/kg highly significantly increasing interleukin (IL)-1, IL-6, IL-15, and β-interferon content in lymphocyte folds 2–47. Zhang et al. [24] reported in an in vitro study on the antifungal activity of citral and cinnamic aldehyde that cinnamic aldehyde exhibits strong antibacterial and antifungal effects, capable of destroying bacterial and fungal structural and functional integrity. The aldehyde group in its structure is hydrophilic and easily adsorbed by hydrophilic groups on fungal surfaces, thereby disrupting cell wall polysaccharide structures and penetrating cell walls. The antibacterial, antifungal, and immune-enhancing functions of cinnamic aldehyde contribute to improved udder health and reduced milk somatic cell count.

3.3 Effects of Cinnamic Aldehyde on Nitrogen Excretion and Nitrogen Apparent Digestibility in Dairy Cows

Ammonia is one of the degradation products of dietary protein and serves as the primary nitrogen source for rumen microbial growth. Rumen protozoa possess strong deamination capacity but cannot utilize NH_3-N for growth and reproduction; therefore, defaunation can reduce NH_3-N concentration and improve rumen nitrogen retention [25]. Benchaar et al. [26] reported in a study on plant essential oils regulating rumen fermentation that the important mechanism by which plant essential oils reduce NH_3-N concentration is the inhibition

of rumen ammonia-producing bacteria. Lin et al. [27] investigated the effects of cinnamon oil and its main components on in vitro rumen fermentation and methane production, suggesting that the reduction in rumen NH_3-N concentration by 200 mg/L cinnamon oil might be due to inhibition of rumen protozoa and gas-producing bacteria. Jin [11] reported that adding 300 and 1,500 mg/L of cinnamic aldehyde significantly reduced NH_3-N concentration during in vitro rumen fermentation. Rumen nitrogen metabolism is also closely related to MCP production. Cinnamic aldehyde can increase the rate of rumen microbial utilization of NH_3-N for MCP synthesis, increase rumen MCP production, reduce NH_3-N loss, and decrease nitrogen emission. As a plant extract, cinnamic aldehyde has functions including preventing feed mold, promoting animal growth, and improving feed utilization efficiency. It can significantly increase nitrogen retention in diets and positively impacts protein synthesis. Cao [16] found that supplementing beef cattle diets with 300, 600, and 900 mg of cinnamic aldehyde daily resulted in a linear increase in feed conversion efficiency, while supplementation at 1,200 mg/day decreased feed conversion efficiency. In this experiment, dietary cinnamic aldehyde supplementation significantly reduced nitrogen excretion in feces and urine and significantly improved nitrogen apparent digestibility.

In conclusion, supplementing dairy cow diets with appropriate levels of cinnamic aldehyde can significantly increase urinary purine derivatives excretion, reduce nitrogen excretion, and improve milk performance. Based on comprehensive consideration of these indicators, the optimal supplementation level of cinnamic aldehyde is 18 g/(d · head) under the conditions of this experiment.

References

[1] Zhou M, Chen Z, Shen S. Research progress on cinnamic aldehyde[J]. Journal of Economic Animal, 2015, 19(1): 1-5, 15.

[2] Zhang Y, Gao Y, Zhu Y, et al. Effects of garlic oil and cinnamic aldehyde complex on production performance and nutrient digestibility of dairy cows[J]. China Feed, 2012(5): 17-20, 23.

[3] Zhou X. Effects of four plant extracts on ammonia emission, growth performance and biochemical indices in broilers[D]. Master's Thesis. Tai'an: Shandong Agricultural University, 2012.

[4] Feng Y, Lu Z. Nutritional Requirements of Dairy Cows and Feed Composition[M]. 3rd ed. Beijing: China Agriculture Press, 2007: 2.

[5] Zhang L. Feed Analysis and Feed Quality Detection Technology[M]. 3rd ed. Beijing: China Agricultural University Press, 2007: 49-74.

[6] Zhu W. Effects of roughage sources on milk protein precursor generation and production performance in dairy cows and its mechanism[D]. Doctoral Disserta-

tion. Hangzhou: Zhejiang University, 2013.

[7] Kohn R A, French K R, Russek-Cohen E. A comparison of instruments and laboratories to measure milk urea nitrogen in bulk-tank milk samples[J]. Journal of Dairy Science, 2004, 87(6): 1848–1853.

[8] Valadares R F D, Broderick G A, Valadares Filho S C, et al. Effect of replacing alfalfa silage with high moisture corn on ruminal protein synthesis estimated from excretion of total purine derivatives[J]. Journal of Dairy Science, 1999, 82(12): 2686–2696.

[9] Qu Y. Application of CNCPS system in dairy production and study on dietary energy-nitrogen balance detection indicators[D]. Doctoral Dissertation. Harbin: Northeast Agricultural University, 2010.

[10] Chen X B, Matuszewski W, Kowalczyk J. Determination of allantoin in biological, cosmetic, and pharmaceutical samples[J]. Journal International, 1996, 79(3): 628–635.

[11] Jin E. In vitro study on effects of plant essential oils on rumen fermentation and methane production[D]. Master' s Thesis. Lanzhou: Gansu Agricultural University, 2013.

[12] Ma T, Diao Q, Deng K. Estimation of rumen microbial protein production using urinary purine derivatives method[J]. Chinese Journal of Animal Nutrition, 2011, 23(1): 10–14.

[13] Wang J. Research Methods in Ruminant Nutrition[M]. Beijing: Modern Education Press, 2011.

[14] Fraser G R, Chaves A V, Wang Y, et al. Assessment of the effects of cinnamon leaf oil on rumen microbial fermentation using two continuous culture systems[J]. Journal of Dairy Science, 2007, 90(5): 2315–2328.

[15] Cardozo P W, Calsamiglia S, Ferret A, et al. Effects of natural plant extracts on ruminal protein degradation and fermentation profiles in continuous culture[J]. Journal of Animal Science, 2004, 82(11): 3230–3236.

[16] Cao A. Application research of cinnamic aldehyde in beef cattle production[J]. Feed China, 2012(16): 37–38.

[17] Yang W Z, Ametaj B N, Benchaar C, et al. Dose response to cinnamaldehyde supplementation in growing beef heifers: ruminal and intestinal digestion[J]. Journal of Animal Science, 2010, 88(2): 680–688.

[18] Yang W Z, Ametaj B N, Benchaar C, et al. Cinnamaldehyde in feedlot cattle diets: Intake, growth performance, carcass characteristics, and blood metabolites[J]. Journal of Animal Science, 2010, 88(3): 1082–1092.

[19] Zhou M, Chen Z, Shen S. Preparation methods and biological functions of cinnamic aldehyde[J]. Chinese Journal of Animal Nutrition, 2014, 26(8): 2040–2045.

[20] Xu X, Cardozo P W, Deng Y, et al. Effects of dietary plant extract supplementation on production performance of early-lactation dairy cows[J]. Journal of Dairy Science and Technology, 2010, 33(3): 139-141.

[21] Kung L Jr, Huber J T. Performance of high producing cows in early lactation fed protein of varying amounts, sources, and degradability[J]. Journal of Dairy Science, 1983, 66(2): 227-234.

[22] Taylor R B, Huber J T, Gomez-Alarcon R A, et al. Influence of protein degradability and evaporative cooling on performance of dairy cows during hot environmental temperatures[J]. Journal of Dairy Science, 1991, 74(1): 243-249.

[23] Lee S H, Lillehoj H S, Jang S I, et al. Cinnamaldehyde enhances in vitro parameters of immunity and reduces in vivo infection against avian coccidiosis[J]. British Journal of Nutrition, 2011, 106(6): 862-869.

[24] Zhang W, Fu Y, Xie X. Study on the antifungal mechanism of citral and cinnamic aldehyde against Aspergillus[J]. Journal of Jiangxi Medical College, 2003, 43(6): 10-13.

[25] McIntosh F M, Williams P, Losa R, et al. Effects of essential oils on ruminal microorganisms and their protein metabolism[J]. Applied and Environmental Microbiology, 2003, 69(8): 5011-5014.

[26] Benchaar C, Calsamiglia S, Chaves A V, et al. A review of plant-derived essential oils in ruminant nutrition and production[J]. Animal Science and Technology, 2008, 145(1/2/3/4): 209-228.

[27] Lin B, Ji M, Liang Q, et al. Effects of cinnamon oil and oregano oil and their main components on in vitro rumen fermentation and methane production[J]. Chinese Journal of Veterinary Science, 2011, 31(2): 279-282, 287.

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