Effects of Compound Enzyme Preparation on Rumen Fermentation, Nutrient Apparent Digestibility, and Production Performance in Lactating Dairy Cows (Postprint)

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Abstract

This experiment aimed to investigate the effects of dietary supplementation with different doses of compound enzyme preparation on rumen fermentation, apparent nutrient digestibility, serum indices, and production performance of lactating dairy cows. Nine lactating Holstein cows with similar body weight, parity [(2.30±0.06) parities], milk yield [(37.00±0.03) kg/d], and days in milk [(90±0.15) d] were selected and randomly divided into 3 groups with 3 cows per group. A 3×3 Latin square design was adopted; the control group was fed a basal diet, while experimental groups I and II were supplemented with 10 and 20 g/(head · d) of compound enzyme preparation on the basis of the basal diet, respectively. Three periods of animal experiments were conducted, each lasting 21 days, including a 14-day preliminary period and a 7-day sampling period. The results showed that: 1) Dietary supplementation with 10 g/(head · d) of compound enzyme preparation significantly increased the concentration of butyrate in rumen fluid of dairy cows ($P<0.05$), but had no significant effect on the concentration of total volatile fatty acids and other volatile fatty acids ($P>0.05$); 2) Compared with the control group, dietary supplementation with 10 g/(head · d) of compound enzyme preparation significantly improved the apparent digestibility of dry matter, crude protein, and neutral detergent fiber in dairy cows ($P<0.05$), and also tended to increase the apparent digestibility of acid detergent fiber ($P=0.06$); 3) Dietary supplementation with 10 and 20 g/(head · d) of compound enzyme preparation significantly increased milk yield of dairy cows ($P<0.05$), and the 10 g/(head · d) group showed increases of 4.85 ($P<0.05$), 0.49 ($P<0.05$), and 0.32 kg/d ($P<0.05$) in 4% fat-corrected milk (4% FCM) yield, milk fat yield, and lactose yield, respectively, compared with the control group; 4) The addition of compound enzyme preparation had

no significant effect on serum indices of dairy cows (P>0.05). In conclusion, dietary supplementation with 10 g/(head · d) of compound enzyme preparation can significantly increase milk yield, milk fat yield, and ruminal butyrate concentration in lactating dairy cows, and the feeding effect is superior to that at the supplementation level of 20 g/(head · d). Taking all factors into consideration, the recommended supplementation level of compound enzyme preparation in the diet of lactating dairy cows is 10 g/(head · d).

Full Text

Effects of Compound Enzyme Preparation on Rumen Fermentation, Nutrient Apparent Digestibility, and Performance of Lactating Dairy Cows

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Abstract: This study investigated the effects of dietary supplementation with different doses of compound enzyme preparation on rumen fermentation, nutrient apparent digestibility, serum parameters, and production performance in lactating dairy cows. Nine Holstein cows with similar body weight, parity [(2.30±0.06) lactations], milk yield [(37.00±0.03) kg/d], and days in milk [(90±0.15) d] were randomly assigned to three groups (n=3 per group) in a 3×3 Latin square design. The control group received a basal total mixed ration (TMR), while trial groups I and II received the basal TMR supplemented with 10 and 20 g/(head · d) of compound enzyme preparation, respectively. The experiment consisted of three periods, each lasting 21 days (14-day adaptation and 7-day sampling). The results showed that: (1) Supplementation with 10 g/(head · d) of compound enzyme preparation significantly increased ruminal butyrate concentration (P<0.05) but had no significant effects on total volatile fatty acids (TVFA) or other individual VFA concentrations (P>0.05). (2) Compared with the control group, 10 g/(head · d) enzyme supplementation significantly improved apparent digestibility of dry matter (DM), crude protein (CP), and neutral detergent fiber (NDF) (P<0.05), with a tendency to increase acid detergent fiber (ADF) digestibility (P=0.06). (3) Both 10 and 20 g/(head · d) enzyme supplementation significantly increased milk yield (P<0.05). Specifically, the 10 g/(head · d) group showed significant increases in 4% fat-corrected milk (4% FCM) yield, milk fat yield, and lactose yield by 4.85 kg/d (P<0.05), 0.49 kg/d (P<0.05), and 0.32 kg/d (P<0.05), respectively, compared with the control group. (4) Enzyme supplementation had no significant effects on serum parameters (P>0.05). In conclusion, dietary supplementation with 10 g/(head ·

d) of compound enzyme preparation significantly improved milk yield, milk fat yield, and ruminal butyrate concentration in lactating cows, with superior efficacy compared to the 20 g/(head · d) dose. Based on these findings, the recommended supplementation rate of compound enzyme preparation for lactating dairy cows is 10 g/(head · d).

Keywords: compound enzyme preparation; lactating dairy cow; rumen fermentation; nutrient apparent digestibility; performance

Feed enzyme preparations are primarily classified as single-enzyme or compound-enzyme preparations. Compound enzyme preparations contain multiple enzymes, typically formulated with one or several single-enzyme preparations as the main components mixed with other enzymes. In the current livestock production environment emphasizing "health, quality, efficiency, and environmental protection," feed enzyme preparations have been widely applied as non-toxic, green additives in compound feeds for animal husbandry.

Initially, exogenous enzyme preparations were applied to monogastric animals such as pigs and poultry, where they improved nutrient digestibility and animal performance through relatively well-understood mechanisms [1-4]. However, research on exogenous enzyme preparations in ruminant nutrition started relatively late. Early scholars believed that enzymes synthesized by ruminants themselves were sufficient for fiber digestion, and that exogenous enzymes would be inactivated by proteolytic bacteria in the rumen, rendering their application unnecessary. Recent studies have confirmed that exogenous enzyme preparations can remain stable in the rumen, reigniting research interest in their supplementation in ruminant diets.

Research has shown that supplementing ruminant diets with compound enzyme preparations primarily containing cellulase and xylanase can disrupt plant cell walls, eliminate anti-nutritional factors, compensate for endogenous enzyme deficiencies, and improve nutrient digestibility, thereby enhancing animal performance [5]. However, the efficacy of compound enzyme preparations is often inconsistent due to factors such as enzyme type, supplementation method, diet composition, and the physiological status of animals [6-9]. With continuous improvements in biotechnology, the application of exogenous compound enzyme preparations in ruminants has become increasingly widespread. Given the variable effects and unclear mechanisms of action in ruminant production practice, this study supplemented a total mixed ration (TMR) with an exogenous compound enzyme preparation primarily containing cellulase, xylanase, and glucanase to investigate its specific effects on rumen fermentation, nutrient apparent digestibility, serum parameters, and production performance in lactating dairy cows. The aim was to expand the database on enzyme application effects and provide insights for rational use and mechanistic studies of compound enzyme preparations.

1.1 Experimental Material

The ruminant-specific compound enzyme preparation used in this experiment was provided by Hunan Youtell Biochemical Co., Ltd., with the following main components: cellulase 3,000 U/g, xylanase 10,000 U/g, -glucanase 5,000 U/g, and pectinase 1,000 U/g.

1.2 Experimental Design

Nine Holstein dairy cows with similar body weight, parity [(2.30±0.06) lactations], milk yield [(37.00±0.03) kg/d], and days in milk [(90.00±0.15) d] were randomly divided into three groups (n=3 per group). A 3×3 Latin square design was employed, with the control group receiving a basal diet and trial groups I and II receiving the basal diet supplemented with 10 and 20 g/(head · d) of compound enzyme preparation, respectively. The experiment consisted of three periods, each lasting 21 days (14-day adaptation and 7-day sampling). Throughout the trial, cows were fed TMR twice daily at 08:00 and 14:00. The pre-weighed enzyme preparation was sprinkled onto the corresponding group' s TMR at 08:00 each day, mixed thoroughly before feeding. Feed intake and refusals were recorded throughout the trial using an automatic feeding system (roughage intake control system, RIC, Netherlands). Cows were milked mechanically three times daily (05:30, 14:00, and 20:00), housed in free-stall barns with ad libitum water access. Daily observations were recorded for feeding behavior, rumination, fecal and urinary conditions, and incidence of mastitis or hoof diseases.

1.3 Experimental Diet

The experiment was conducted at the Zhongdi Elite Dairy Science and Technology Park in Shunyi District, Beijing. A TMR formulated with corn, corn silage, and alfalfa as main ingredients served as the basal diet. The composition and nutrient levels of the basal diet are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis) %

Item	Content
Ingredients (air-dry basis)	
Corn silage	
Imported alfalfa	
Domestic alfalfa	
Oat grass	
Chinese wildrye	
Corn	
Wheat grain	
Extruded soybean	
Soybean meal	

Item	Content
Molasses	
Steam-flaked corn	
Cottonseed	
Soybean hulls	
Rumen-pass fatty acid[1])	
Yeast culture	
Mycotoxin removement agent[2])	
NaCl	
Limestone	
NaHCO	
CaHPO	
Mineral-vitamin premix[3])	
MgO	
Total	
Nutrient levels (DM basis))	
NEL/(MJ/kg)	
CP	
NDF	
ADF	
EE	
Ash	
Ca	

[1]) Rumen-pass fatty acid purchased from Berg-Schmidt Co., Germany.
[2]) Mycotoxin removement agent purchased from Biomin Co., Austria.
[3]) Mineral-vitamin premix provided the following per kg of diet: VA 1,000,000 IU, VD 280,000 IU, VE 10,000 IU, nicotinic acid 1,000 mg, Cu 3,250 mg, Mn 4,800 mg, Zn 12,850 mg, I 140 mg, Se 150 mg, Co 110 mg.
) NEL was a calculated value [NEL (MJ/kg) = 0.0551 × DE (MJ/kg) - 0.0946], while other nutrient levels were measured values.

1.4 Sampling and Analysis Methods

1.4.1 Feed Sample Collection and Analysis Daily feed intake and refusals were recorded using the automatic feeding monitoring system to calculate dry matter intake (DMI). Feed samples were collected weekly, dried at 65°C, equilibrated, and processed into air-dried samples for storage. Following the methods described by Zhang Liying [10], feed samples were analyzed for dry matter (DM), then for crude protein (CP), ether extract (EE), ash, neutral detergent fiber (NDF), acid detergent fiber (ADF), calcium (Ca), and phosphorus (P) on a DM basis. Acid-insoluble ash (AIA) content was also determined for calculating nutrient apparent digestibility.

1.4.2 Milk Yield and Composition Measurement Daily milk yield was recorded during each sampling period, and 4% fat-corrected milk (4% FCM) yield was calculated. On days 1 and 2 of each sampling period, milk samples were collected and pooled in a 4:3:3 ratio (morning:afternoon:evening) into 50 mL containers with potassium dichromate preservative. Samples were immediately sent to the Beijing Dairy Cattle Center for analysis using a MilkoScan 605 multifunctional milk composition analyzer (Foss Electric, Hillerod, Denmark) to determine milk fat percentage, milk protein percentage, lactose percentage, somatic cell count, and milk urea nitrogen content.

1.4.3 Rumen Fluid Collection and Analysis On days 3 and 4 of each sampling period, rumen contents (50 mL) were collected via oral intubation at 2-hour intervals (0, 2, 4, 6, and 8 h) after morning feeding (08:00). After filtration through four layers of cheesecloth, pH was measured immediately. Samples were then centrifuged at 1,500×g for 15 min, and the supernatant was collected and stored at -20°C in two plastic bottles for determination of ammonia nitrogen (NH -N) and volatile fatty acid (VFA) concentrations. NH -N concentration was determined by the phenol-hypochlorite colorimetric method using a UV spectrophotometer (UV-2600, Unico, Shanghai), while VFA concentration was analyzed by gas chromatography (Agilent 6890N, Beijing Beifen Tianpu Instrument Technology Co., Ltd.).

1.4.4 Blood Sample Collection and Analysis On day 7 of each sampling period, 10 mL of blood was collected from the tail vein using vacuum tubes (Shandong Aosite Medical Devices Co., Ltd.) before morning feeding. Blood samples were centrifuged immediately at 1,500×g for 15 min, and serum was aliquoted into 1.5 mL tubes and stored at -20°C. Serum samples were sent to Beijing Labtech Technology Development Co., Ltd. for colorimetric determination of glucose (GLU), triglyceride (TG), total cholesterol (TC), free fatty acid (FFA), -hydroxybutyric acid (BHBA), urea nitrogen (UN), total protein (TP), and albumin (ALB).

1.4.5 Fecal Sample Collection and Analysis During days 5–7 of each sampling period, fecal samples were collected continuously 12 times via rectal grab sampling (300–500 g per sample). Sampling times were 04:00, 09:00, 14:00, and 19:00 on day 5; 05:00, 10:00, 15:00, and 20:00 on day 6; and 06:00, 11:00, 17:00, and 22:00 on day 7. After the final collection, fecal samples from each cow were thoroughly mixed, and approximately 200 g was taken and mixed with 10% tartaric acid (1/4 of fecal weight) before drying to prepare air-dried samples. These were analyzed for nutrient and AIA content to calculate apparent digestibility using the formula from Zhong et al. [11]:

$$\textbf{Nutrient apparent digestibility} = [1 - (Ad \times Nf) / (Af \times Nd)] \times 100$$

Where Ad (g/kg) and Af (g/kg) represent AIA content in diet and feces, respectively; Nd (g/kg) and Nf (g/kg) represent the corresponding nutrient content

in diet and feces, respectively.

1.5 Statistical Analysis

Experimental data were initially processed in Excel 2007 and analyzed using the ANOVA model in SPSS 19.0. Duncan' s multiple range test was applied for post-hoc comparisons, with significance set at $P<0.05$. Results are expressed as mean ± standard error (SE).

2.1 Effects of Compound Enzyme Preparation on Rumen pH, NH -N, and VFA Concentrations

As shown in Table 2 , compared with the control group, trial group I exhibited significantly higher ruminal butyrate concentration ($P<0.05$). Additionally, trial group I showed tendencies for increased acetate concentration and decreased NH -N concentration, though these differences were not statistically significant ($P>0.05$). Rumen pH, TVFA concentration, propionate concentration, and acetate/propionate ratio were not significantly affected by enzyme supplementation ($P>0.05$).

Table 2 Effects of compound enzyme preparation on pH and concentrations of NH -N and VFA in rumen fluid of lactating cows

Item	Control	Trial Group I	Trial Group II	P-value
pH	6.53±0.04	6.51±0.06	6.52±0.06	
NH -N (mg/mL)	13.63±0.27	11.97±0.24	12.40±0.19	
Acetate (mmol/L)	58.63±2.09	61.50±1.95	59.60±1.40	
Propionate (mmol/L)	25.57±1.41	25.04±1.38	25.87±1.02	
Butyrate (mmol/L)	10.78±0.47	13.86±0.75	11.01±0.34	<0.05
Acetate/Propionate	2.29±0.56	2.46±0.63	2.30±0.44	
TVFA (mmol/L)	94.99±5.49	100.41±4.89	96.48±2.11	

In the same row, values with no letter or the same letter superscripts indicate no significant difference ($P>0.05$), while different lowercase letters indicate significant difference ($P<0.05$). The same applies below.

Figure 1 [Figure 1: see original paper] illustrates the postprandial changes in ruminal pH and NH -N concentration at 0, 2, 4, 6, and 8 h after feeding. The pH dynamics were similar across groups, gradually decreasing from 0 h post-feeding, reaching the lowest value around 6 h, then increasing again. NH -N concentration patterns were also consistent among groups, peaking at 2 h post-feeding, then gradually decreasing until the second feeding.

2.2 Effects of Compound Enzyme Preparation on Nutrient Apparent Digestibility

As shown in Table 3 , supplementation with 10 g/(head · d) of compound enzyme preparation significantly improved apparent digestibility of DM, CP, and NDF (P<0.05). Although enzyme supplementation did not significantly affect ADF digestibility (P>0.05), it showed a tendency to improve it. While differences between trial groups I and II were not significant (P>0.05), all nutrient digestibility values were numerically higher in trial group I than in trial group II.

Table 3 Effects of compound enzyme preparation on nutrient apparent digestibility of lactating cows

Item	Control	Trial Group I	Trial Group II	P-value
DM	69.34±0.04	71.35±0.06	69.96±0.05	<0.05
CP	73.45±0.28	76.12±0.52	75.65±0.19	<0.05
NDF	58.08±1.25	62.26±0.86	61.18±1.47	<0.05
ADF	56.12±1.84	58.61±1.54	56.53±1.48	

2.3 Effects of Compound Enzyme Preparation on DMI, Milk Yield, and Milk Composition

As shown in Table 4 , no significant differences in DMI were observed among groups (P>0.05). However, enzyme supplementation significantly increased milk yield (P<0.05), with similar magnitude of improvement in both trial groups compared with the control. Trial group I showed significant improvements not only in milk yield and milk fat percentage (P<0.05) but also in 4% FCM yield, milk fat yield, and lactose yield, which increased by 4.85 kg/d (P<0.05), 0.49 kg/d (P<0.05), and 0.32 kg/d (P<0.05), respectively, compared with the control group. Other milk components did not differ significantly among groups (P>0.05).

Table 4 Effects of compound enzyme preparation on DMI, milk production, and milk composition of lactating cows

Item	Control	Trial Group I	Trial Group II	P-value
DMI (kg/d)	23.09±0.76	23.61±0.50	23.62±0.58	
Milk yield (kg/d)	41.27±1.36	42.21±1.08	42.24±1.03	<0.05
4% FCM (kg/d)	37.92±1.35	42.77±1.70	40.57±1.63	<0.05

Item	Control	Trial Group I	Trial Group II	P-value
Feed efficiency				
Milk fat percentage (%)	3.27±0.15	4.03±0.17	3.76±0.29	<0.05
Milk fat yield (kg/d)	1.25±0.10	1.74±0.12	1.55±0.14	<0.05
Milk protein percentage (%)	3.15±0.12	3.29±0.10	3.30±0.13	
Milk protein yield (kg/d)	1.19±0.06	1.34±0.08	1.41±0.08	
Lactose percentage (%)	4.81±0.12	5.00±0.07	4.86±0.16	
Lactose yield (kg/d)	1.82±0.06	2.14±0.09	1.96±0.09	<0.05
UN (mg/dL)	16.61±0.79	14.79±1.32	15.70±1.52	
Somatic cell count ($\times 10^3$ mL ^{1})	37.78±6.98	35.88±6.56	38.13±6.67	

2.4 Effects of Compound Enzyme Preparation on Serum Parameters

As shown in Table 5 , enzyme supplementation tended to increase serum FFA and BHBA concentrations but without reaching statistical significance (P>0.05).

Other serum parameters were also not significantly affected by enzyme supplementation (P>0.05), indicating that exogenous enzyme preparation did not significantly impact cow health status.

Table 5 Effects of compound enzyme preparation on serum indexes of lactating cows

Item	Control	Trial Group I	Trial Group II	P-value
GLU (mmol/L)	4.93±0.17	4.98±0.14	4.86±0.08	
TP (g/L)	75.22±1.86	74.78±1.87	73.33±2.31	
ALB (g/L)	35.56±0.56	34.78±0.94	35.11±0.31	
GLB (g/L)	39.66±0.60	40.00±0.84	38.22±0.41	
TG (mmol/L)	0.55±0.02	0.53±0.04	0.51±0.02	
FFA (mol/mL)	71.05±13.32	97.78±22.51	93.81±25.71	
BHBA (mmol/L)	0.63±0.87	0.84±0.19	0.81±0.17	
TC (mmol/L)	8.87±0.78	8.97±0.58	8.76±0.70	
UN (mmol/L)	5.78±0.37	5.88±0.26	5.89±0.29	

3.1 Effects of Compound Enzyme Preparation on Rumen Fermentation in Lactating Cows

Ruminal pH is a crucial indicator reflecting rumen fermentation status, typically fluctuating within a certain range. Feng Yanglian [12] reported that the normal range of ruminal pH is 6.0–7.0. In this study, ruminal pH showed minimal variation in both enzyme-supplemented and control groups, with all values within the normal range. Previous research has shown that diet structure and nutrient levels are the fundamental factors affecting ruminal pH [13]. Since all groups received the same TMR throughout the trial, no significant effects on ruminal pH were expected.

Volatile fatty acids (VFAs) produced from carbohydrate fermentation by rumen microorganisms are important indicators of rumen health, providing 70–80% of energy requirements for ruminants. Acetate, propionate, and butyrate account for approximately 95% of TVFA concentration. Propionate is the primary precursor for gluconeogenesis, while acetate and glucose exhibit substantial interdependence for milk fat synthesis in dairy cows [14]. Arriola et al. [15] reported that dietary fibrolytic enzyme supplementation increased ruminal TVFA concentration but decreased the acetate/propionate ratio. Chung et al. [16] found no significant changes in ruminal TVFA, NH -N concentration, or pH when supplementing a fibrolytic enzyme preparation. Li Yanling et al. [17] observed no significant differences in TVFA concentration or acetate/propionate ratio in fermentation fluid after adding a cellulase- and xylanase-based compound enzyme, except for isovalerate. In contrast, our results showed that enzyme supplementation at 10 g/(head · d) significantly increased butyrate concentration without affecting other VFA concentrations. Ruminal butyrate is primarily synthesized

from acetate through the reverse reaction of fatty acid -oxidation under microbial action and is less affected by other factors. Our study found that acetate concentration tended to increase in both trial groups compared with the control, suggesting that elevated acetate may have promoted butyrate synthesis via enhanced reverse -oxidation, though the specific mechanism requires further investigation.

Ruminal microbial growth requires an optimal NH -N concentration, with reported tolerance ranges of 6–30 mg/dL. Ruminal NH -N concentration is determined by both protein degradation in the rumen and microbial ammonia utilization rates. In this study, NH -N concentrations in all groups were within the normal range with similar variation patterns. Numerically, both enzyme doses tended to decrease ruminal NH -N concentration, possibly due to enhanced microbial ammonia utilization. Li Yanling et al. [17] also observed numerically reduced NH -N concentration in fermentation fluid, speculating that microbial NH -N utilization had increased, which aligns with our findings.

3.2 Effects of Compound Enzyme Preparation on Nutrient Apparent Digestibility in Lactating Cows

Initially, some researchers believed that exogenous cellulase-based compound enzyme preparations would be inactivated by proteolytic bacteria in the rumen. However, studies have confirmed that exogenous enzymes can remain stable in the ruminal environment [18-19]. The stability of exogenous enzymes in the rumen may be related to enzyme glycosylation, as some enzymes produced by specific ruminal microbial flora can prevent hydrolysis of exogenous enzymes even without glycosylation [20]. Consequently, most studies have reported positive effects of enzyme supplementation on feed digestibility [21-24].

Under normal conditions, exogenous enzymes constitute only a small portion of total ruminal enzymes, making it difficult to attribute improvements in fiber digestion solely to direct enzymatic action. Therefore, some researchers have speculated that exogenous enzymes may function synergistically with endogenous enzymes [25] or enhance microbial attachment to feed particles [18]. Other studies have found that exogenous enzyme supplementation significantly increased fiber-degrading bacterial populations in sheep and dairy cows, suggesting that altered ruminal microbial populations may represent a potential mode of action [26-27]. Our results demonstrated that 10 g/(head · d) enzyme supplementation significantly improved apparent digestibility of DM, CP, and NDF, with a tendency to promote ADF digestion. The 20 g/(head · d) dose also improved nutrient digestibility. This may be attributed to synergistic effects among cellulase, xylanase, glucanase, and pectinase in the compound preparation, which maximally reduces feed viscosity, disrupts plant cell walls (especially non-starch polysaccharides like cellulose, xylan, and pectin), enhances feed nutritional value during rumen fermentation, releases nutrients for animal digestion and absorption, and improves NDF digestibility, thereby increasing overall feed digestibility. This explains why cellulase-based compound enzyme supplementation can simulta-

neously improve digestibility of both fiber and non-fiber components. However, excessive enzyme supplementation may compete with ruminal microbes for feed binding sites, potentially impairing endogenous enzyme attachment and digestion. In our study, the 20 g/(head·d) dose was less effective than the 10 g/(head·d) dose, highlighting the importance of considering appropriate supplementation levels for optimal efficacy.

3.3 Effects of Compound Enzyme Preparation on Production Performance of Lactating Cows

Numerous studies have confirmed that appropriate enzyme supplementation can improve animal performance, though reports on its effects on milk yield and composition in dairy cows have been inconsistent. Yang et al. [21] reported that supplementing alfalfa-based diets with cellulase- and xylanase-based enzymes significantly increased milk yield in a dose-dependent manner but had minimal effects on milk composition. In contrast, Bowman et al. [28] and Eun et al. [29] found positive effects on milk fat and protein percentages. Recent domestic studies have also shown that different enzyme doses can increase milk yield to varying degrees without significantly affecting milk composition. Du Renrang [30] reported increased milk yield but unchanged milk composition when supplementing dairy cow concentrate with four different enzyme doses. Liu Yunbo et al. [31] observed improvements in both milk yield and milk fat percentage when adding compound enzyme to corn silage and wheat straw-based diets. Wang Chaoli et al. [32] found that 30 g/(head·d) enzyme supplementation significantly increased milk yield without affecting milk composition. The variable results may be attributed to factors such as enzyme type, dosage, and supplementation method.

Our study found that enzyme supplementation did not significantly affect DMI but significantly increased milk yield. The 10 g/(head·d) group showed significant improvements in 4% FCM yield, milk fat percentage, milk fat yield, and lactose yield, consistent with findings from Zhou Xiaojuan et al. [33] and Du Ruiping et al. [34]. DMI is influenced by multiple factors including animal individuality, environment, and diet composition [35]. Since all groups received the same TMR under similar environmental conditions with comparable animal characteristics, no significant differences in DMI were observed. However, by improving rumen fermentation and overall nutrient digestibility, enzyme supplementation enhanced feed conversion efficiency, thereby increasing milk yield and improving milk composition.

3.4 Effects of Compound Enzyme Preparation on Serum Parameters of Lactating Cows

Serum parameters are commonly used to assess cow health and metabolic status. Serum glucose content reflects the dynamic balance of glucose absorption, transport, and metabolism, typically maintaining constant values. Normal serum

total protein and albumin concentrations in dairy cows range from 62–82 g/L and 28–39 g/L, respectively, generally showing similar trends [36]. Blood urea nitrogen, derived from tissue protein catabolism and ruminal ammonia absorption, is the end product of protein metabolism and remains relatively stable, influenced by dietary nitrogen intake and endogenous nitrogen secretion. Our study found no significant differences in serum glucose, total protein, albumin, or urea nitrogen among groups, likely due to the strong homeostatic regulation in ruminants. Li Kui et al. [37] reported similar findings in Angus beef cattle, where enzyme supplementation affected these parameters numerically but not statistically.

Free fatty acids, derived from triglyceride mobilization and lipolysis in adipose tissue, are widely involved in metabolic cycles and constantly fluctuate. -hydroxybutyric acid is primarily synthesized from free fatty acid oxidation in the liver and from butyrate absorbed across the rumen wall [38-39], serving as a precursor for milk fat synthesis in ruminants. Our study found that enzyme supplementation increased serum FFA and BHBA concentrations to varying degrees, indicating enhanced body fat mobilization. Additionally, the increased ruminal butyrate concentration observed in our study may have contributed to elevated BHBA, as most butyrate is converted to BHBA during absorption through the rumen wall, further confirming the potential of enzyme supplementation to improve milk fat percentage.

Conclusion

Dietary supplementation with 10 g/(head · d) of compound enzyme preparation significantly improved apparent digestibility of NDF, CP, and DM, promoted ADF digestion, and consequently increased milk yield and improved milk composition, with superior efficacy compared to the 20 g/(head · d) dose. Based on comprehensive evaluation, the recommended supplementation rate of compound enzyme preparation for lactating dairy cows is 10 g/(head · d).

References

[1] Fu Shuiguang, Wang Zirui, You Jinming, et al. Effects of compound enzyme preparation on growth performance and nutrient digestibility of weaned piglets [J]. Feed Industry, 2010, 31(7): 40–42.
[2] Sun Xiaoming, Du Dewei, Meng Xianmei. Application of compound enzyme preparation in growing-finishing pig production [J]. Journal of Jilin Business and Technology College, 2012, 28(2): 107–109.
[3] He Yanfang. Mechanism of compound enzyme preparation and its application in broiler production [J]. Chinese Journal of Traditional Veterinary Science, 2015(11): 12–13.
[4] Zhang Qin. Effects of compound enzyme preparation on production performance and blood biochemical indices of broilers [J]. Journal of Anhui Agricultural Sciences, 2012, 40(17): 9285–9287.

[5] Xie Xiangxue, Du Hongfang, Chen Shuqin, et al. Application and prospects of exogenous enzyme preparations in ruminants [J]. Chinese Journal of Animal Nutrition, 2016, 28(4): 1011-1019.
[6] Gao Min, Lu Dexun. Application of enzyme preparations in ruminants [J]. China Feed, 2004(20): 5-7.
[7] Liu Ruiling, Li Keguang. Application of active compound enzyme preparation in dairy production [J]. Journal of Animal Science and Veterinary Medicine, 2012, 31(4): 51-53.
[8] Wang Hongyun, Gao Zhanfeng, Fu Cai. Application of exogenous enzyme preparations in ruminants [J]. Feed Industry, 2006, 27(2): 23-25.
[9] Liu Ruiling, Wang Xiangzu. Study on compound enzyme preparation in dairy production [J]. Feed Review, 2016(8): 6-8.
[10] Zhang Liying. Feed Analysis and Feed Quality Detection Technology [M]. 3rd ed. Beijing: China Agricultural University Press, 2007: 67-68.
[11] Zhong R Z, Li J G, Gao Y X, et al. Effects of substitution of different levels of steam-flaked corn for finely ground corn on lactation and digestion in early lactation dairy cows [J]. Journal of Dairy Science, 2008, 91(10): 3931-3937.
[12] Feng Yanglian. Ruminant Nutrition [M]. Beijing: Science Press, 2004: 138-141.
[13] Wang Qingli, Tian Lanying, Zhao Renyi, et al. Factors affecting rumen pH in dairy cows [J]. Henan Journal of Animal Husbandry and Veterinary Medicine: Comprehensive Edition, 2008, 29(19): 36-37.
[14] Hao Zhimin, Wang Jing, Zhu Liangjing, et al. Effects of acetate/propionate ratio on in vitro fermentation and protein production by rumen microbes [J]. Guangdong Feed, 2011, 20(8): 20-24.
[15] Arriola K G, Kim S C, Staples C R, et al. Effect of fibrolytic enzyme application to low and high-concentrate diets on the performance of lactating dairy cows [J]. Journal of Dairy Science, 2011, 94(2): 832-841.
[16] Chung Y H, Zhou M, Holtshausen L, et al. A fibrolytic enzyme additive for lactating Holstein cow diets: ruminal fermentation, rumen microbial populations, and enteric methane emissions [J]. Journal of Dairy Science, 2011, 95(3): 1419-1427.
[17] Li Yanling, Zhang Min, Chai Jianmin, et al. Effects of exogenous compound enzyme preparation on in vitro rumen fermentation and milk performance of dairy cows [J]. Chinese Journal of Animal Nutrition, 2015, 27(9): 2911-2919.
[18] Morgavi D P, Newbold C J, Beever D E, et al. Stability and stabilization of potential feed additive enzymes in rumen fluid [J]. Enzyme and Microbial Technology, 2000, 26(2/3/4): 171-177.
[19] Zhang Qin, Mao Shengyong, Zhu Weiyun. Application and mechanism of exogenous enzymes in ruminant production [J]. Animal Husbandry and Veterinary Medicine, 2007, 39(5): 55-58.
[20] Fontes C M G A, Hall J, Hirst B H, et al. The resistance of cellulases and xylanases to proteolytic inactivation [J]. Applied Microbiology and Biotechnology, 1995, 43(1): 52-57.
[21] Yang W Z, Beauchemin K A, Rode L M. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows [J]. Journal

of Dairy Science, 1999, 82(2): 391–403.
[22] Álvarez G, Pinos-Rodríguez J M, Herrera J G, et al. Effects of exogenous fibrolytic enzymes on ruminal digestibility in steers fed high fiber rations [J]. Livestock Science, 2009, 121(2/3): 150–154.
[23] Xu Xuewen, Liu Zhengya, Tian Wen, et al. Effects of different compound enzyme preparations on production performance and nutrient digestibility of dairy cows [J]. Cereal and Feed Industry, 2012, 12(11): 51–54.
[24] Wang Zhaozhong, Shang Youan. Effect analysis of cellulase compound enzyme on digestibility of dairy cows [J]. Breeding and Feed, 2008(5): 61–62.
[25] Liu Dasen, Ju Tao, Yang Fang, et al. Research progress on application of cellulase in ruminant feed [J]. Journal of Northeast Agricultural University, 2011, 42(6): 7–11.
[26] Giraldo L A, Tejido M L, Ranilla M J, et al. Influence of direct-fed fibrolytic enzymes on diet digestibility and ruminal activity in sheep fed a grass hay-based diet [J]. Journal of Animal Science, 2008, 86(7): 1617–1623.
[27] Nsereko V L, Beauchemin K A, Morgavi D P, et al. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows [J]. Canadian Journal of Microbiology, 2002, 48(1): 14–20.
[28] Bowman G R, Beauchemin K A, Shelford J A. The proportion of the diet to which fibrolytic enzymes are added affects nutrient digestion by lactating dairy cows [J]. Journal of Dairy Science, 2002, 85(12): 3420–3429.
[29] Eun J S, Beauchemin K A. Effects of a proteolytic feed enzyme on intake, digestion, ruminal fermentation, and milk production [J]. Journal of Dairy Science, 2005, 88(6): 2140–2153.
[30] Du Renrang. Study on effects of dietary enzyme levels on milk yield and composition of dairy cows [D]. Master' s thesis. Yangling: Northwest A&F University, 2007: 22–28.
[31] Liu Yunbo, Li Changsheng, Jiang Shu. Effects of compound enzyme preparation on milk performance of dairy cows [J]. Feed Research, 2002(11): 33–34.
[32] Wang Chaoli, Wang Shujie, Xu Cunzhu, et al. Effects of ruminant-specific compound enzyme on production performance of dairy cows [J]. China Dairy Cattle, 2015(16): 14–17.
[33] Zhou Xiaojuan, Bai Yueyu, Liang Xu, et al. Effects of compound enzyme on milk yield and composition of dairy cows [J]. China Dairy Cattle, 2014(1): 5–7.
[34] Du Ruiping, Duan Zhiyong, Lao Ye, et al. Effects of compound enzyme preparation on milk yield and quality of dairy cows [J]. Feed Industry, 2008, 29(17): 41–43.
[35] Wu Qiujue, Xu Tingsheng, Huang Dingzhou. Factors affecting dry matter intake in ruminants [J]. Feed and Animal Husbandry, 2006(12): 31–34.
[36] Wang Jundong, Liu Zongping. Veterinary Clinical Diagnostics [M]. Beijing: China Agriculture Press, 2004.
[37] Li Kui, Yang Liang, Zhang Wenju, et al. Effects of compound enzyme preparation on production performance and serum biochemical indices of Angus beef cattle [J]. Feed Review, 2016(4): 5–8.

[38] Roche J R, Sheahan A J, Chagas L M, et al. Short communication: change in plasma ghrelin in dairy cows following an intravenous glucose challenge [J]. Journal of Dairy Science, 2008, 91(3): 1005-1010.
[39] Andersson L, Lundström K. Effect of feeding silage with high butyric acid content on ketone body formation and milk yield in postparturient dairy cows [J]. Zentralblatt Für Veterinärmedizin, 1985, 32(1-10): 15-23.

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