

Effects of Dietary Supplementation with Compound Enzyme Preparation on Rumen Fermentation, Apparent Digestibility of Nutrients, and Production Performance in Dairy Cows: Post-print

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Date: 2018-12-25T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of dietary supplementation with different doses of compound enzyme preparation on rumen fermentation, nutrient apparent digestibility, serum biochemical indices, and production performance of dairy cows. Thirty-two early-lactation Holstein dairy cows with similar body weight, parity, days in milk, and milk yield were selected and allocated to 4 groups using a randomized block design, with 8 cows per group. The control group was fed the basal diet without compound enzyme preparation, while the experimental groups were fed the basal diet supplemented with 0.10%, 0.15%, and 0.20% compound enzyme preparation, respectively. The experiment consisted of a 10-day preliminary period followed by a 56-day experimental period. The results showed: 1) Compared with the control group, dietary supplementation with 0.15% compound enzyme preparation significantly increased rumen fluid total volatile fatty acid content ($P < 0.05$); enzyme supplementation tended to increase rumen fluid acetic acid content ($P = 0.08$), while having no significant effect on other volatile fatty acids, ammonia nitrogen, and microbial protein content ($P > 0.05$), and showed a tendency to decrease pH ($P = 0.08$). 2) Compared with the control group, dietary supplementation with compound enzyme preparation significantly improved the apparent digestibility of dry matter and neutral detergent fiber ($P < 0.05$); supplementation with 0.20% compound enzyme preparation significantly improved the apparent digestibility of organic matter ($P < 0.05$). 3) Compared with the control group, dietary supplementation with compound enzyme preparation showed an increasing trend in serum total protein content ($P = 0.09$) and a decreasing trend in urea nitrogen content ($P = 0.07$), while having no significant effect on other serum biochemical indices

($P > 0.05$). 4) Compared with the control group, dietary supplementation with 0.10% and 0.15% compound enzyme preparation significantly improved feed conversion efficiency ($P < 0.05$); the 3.5% fat-corrected milk yield of the 0.10% and 0.15% groups increased by 3.88 and 4.27 kg/d, respectively ($P < 0.05$), and the 0.15% group increased milk fat percentage by 12.70% ($P < 0.05$). Under the conditions of this experiment, dietary supplementation with compound enzyme preparation helped to improve feed conversion efficiency, rumen fermentation, and production performance, with the addition level of 0.15% showing better effects.

Full Text

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

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Abstract

This experiment was conducted to investigate the effects of different doses of compound enzyme preparation on rumen fermentation, apparent digestibility of nutrients, serum biochemical indices, and performance of lactating cows. Thirty-two healthy Holstein cows in early lactation were selected based on similar body weight, parity, lactation days, and milk yield, and allocated to four groups using a randomized block design ($n=8$ per group). Cows in the control group were fed a basal diet without enzyme supplementation, while those in the experimental groups received the basal diet supplemented with 0.10%, 0.15%, and 0.20% compound enzyme preparation, respectively. The preliminary period lasted 10 days, followed by a 56-day formal experimental period. The results showed that: (1) Compared with the control group, dietary supplementation with 0.15% compound enzyme preparation significantly increased total volatile fatty acid (TVFA) content in rumen fluid ($P<0.05$), while enzyme addition tended to increase acetic acid content ($P=0.08$) and decrease pH ($P=0.09$), but had no significant effects on other volatile fatty acids, ammonia nitrogen, or microbial protein content ($P>0.05$). (2) Enzyme supplementation significantly improved the apparent digestibility of dry matter (DM) and neutral detergent

fiber (NDF) ($P < 0.05$), and 0.20% enzyme addition significantly increased organic matter (OM) digestibility ($P < 0.05$). (3) Enzyme addition tended to increase serum total protein content ($P = 0.09$) and decrease urea nitrogen content ($P = 0.07$), with no significant effects on other serum biochemical indices ($P > 0.05$). (4) The 0.10% and 0.15% enzyme groups significantly improved feed conversion ratio (FCR) ($P < 0.05$), increased 3.5% fat-corrected milk yield by 3.88 and 4.27 kg/d, respectively ($P < 0.05$), and the 0.15% group increased milk fat percentage by 12.70% ($P < 0.05$). Under the conditions of this experiment, dietary supplementation with compound enzyme preparation improved feed conversion, rumen fermentation, and lactation performance, with 0.15% being the optimal inclusion level.

Keywords: compound enzyme preparation; dairy cows; apparent digestibility; performance; serum biochemical indices

Introduction

Low dietary utilization has long been a major factor limiting performance improvement in dairy production. Exogenous compound enzyme preparations, extracted from biological sources with enzymatic properties, have rapidly become a research hotspot in feed additives due to their green, safe, and efficient characteristics. Initially applied in monogastric animals such as pigs and poultry to improve feed utilization and performance, enzyme application in ruminants started relatively later due to concerns about rumen microbial degradation and inactivation. However, recent studies have confirmed that exogenous enzymes can remain stable in the rumen, renewing research interest in their application for ruminants. While some studies have reported that enzyme supplementation improves dietary digestibility, increases milk yield, and enhances milk quality, others have found no significant effects on dairy cow performance. The efficacy of enzyme preparations depends on factors including enzyme type, supplementation method, dosage, and production process. Single enzyme preparations are limited by their narrow function, making compound preparations more common. Current research on compound enzymes in dairy cows has focused primarily on cellulase and xylanase for degrading plant cell wall components, with limited studies on formulations combining cellulase, xylanase, mannanase, α -glucanase, pectinase, and neutral protease. This experiment utilized a compound enzyme preparation made from genetically modified *Trichoderma reesei* cellulase (second generation) combined with xylanase, mannanase, α -glucanase, pectinase, and neutral protease to investigate its effects on rumen fermentation, nutrient apparent digestibility, serum biochemical indices, and performance, while determining the optimal supplementation level for lactating cows.

Materials and Methods

1.1 Experimental Materials

The exogenous compound enzyme preparation used in this experiment was provided by Ningxia Xiasheng Industrial Co., Ltd., with main components including cellulase (15,000 U/g), xylanase (100,000 U/g), α -glucanase (280,000 U/g), mannanase (2,500 U/g), pectinase (60,000 U/g), and neutral protease (2,000 U/g).

1.2 Experimental Animals and Design

Thirty-two healthy Holstein cows in early lactation were selected based on similar body weight, parity, lactation days, and milk yield, and randomly allocated to four groups using a randomized block design (n=8 per group, each cow serving as a replicate). The control group received a basal diet without enzyme supplementation, while experimental groups received the basal diet supplemented with 0.10%, 0.15%, and 0.20% compound enzyme preparation, respectively. The enzyme preparation was first mixed thoroughly with concentrate according to the designed inclusion levels, then combined with roughage to form a total mixed ration (TMR). The preliminary period lasted 10 days, followed by a 56-day formal experimental period. Basic information on experimental cows is presented in Table 1 .

1.3 Experimental Diet and Management

The basal diet was formulated according to NRC (2001) nutrient requirements for high-yielding dairy cows, with composition and nutrient levels shown in Table 2 . Cows were housed in a free-stall barn with bedding, with free access to feed and water. Feeding was conducted using an Insentec automatic feeding system (Netherlands) with TMR provided twice daily at 07:30 and 14:30. Milking was performed using a BouMatic automatic milking system (USA) three times daily at 06:30, 14:00, and 20:30.

1.4 Measurements

1.4.1 Feed Intake and Related Indices Daily feed intake was automatically recorded using the Insentec system. Diet samples were collected twice weekly during the experimental period to determine dry matter (DM) content. Dry matter intake (DMI) and feed conversion ratio (FCR) were calculated based on daily intake and milk yield records. DM, crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), and crude ash contents were determined according to national standards [10-13], with organic matter (OM) content calculated from crude ash content.

1.4.2 Rumen Fluid Collection and Analysis On day 54 of the formal period, rumen fluid (100 mL) was collected 1 hour after morning feeding using a

stomach tube sampler (MDW-15, Shanghai Guidi Co.) via the oral cavity. After filtration through four layers of gauze, pH was immediately measured using a pH meter (SevenGo™ pH-SG2, Shanghai Zhanxing Instrument Technology Co., Ltd.). Samples were then aliquoted into 10 mL centrifuge tubes and stored at -20°C for determination of ammonia nitrogen (NH -N), microbial crude protein (MCP), and volatile fatty acids (VFA). NH -N content was determined by the phenol-hypochlorite colorimetric method [14] using a microplate reader (Thermo Electron Varioskan Flash, Shanghai Fuze Trading Co., Ltd.). MCP content was measured by the purine base method [15] using the same microplate reader. VFA content was analyzed by gas chromatography (Agilent-6890N, Beijing Beifen Tianpu Instrument Technology Co., Ltd.).

1.4.3 Blood Sample Collection and Analysis On day 54 of the formal period, blood samples (10 mL) were collected from the tail vein using vacuum tubes 1 hour before morning feeding. Samples were centrifuged at 2,000×g for 10 minutes (Himac-CR22G), and serum was aspirated into 2 mL tubes and stored at -20°C. Serum samples were sent to Huaying Biotechnology Co., Ltd. for analysis of urea nitrogen (UN), glucose (GLU), creatinine (CR), total protein (TP), albumin (ALB), total cholesterol (TC), and γ -hydroxybutyric acid (BHBA) using a Mindray BS300 biochemical analyzer (Beijing Mairun Medical Equipment Co., Ltd.).

1.4.4 Milk Yield and Composition Milk yield was recorded using the BouMatic automatic milking system, and 3.5% fat-corrected milk yield was calculated. Fresh milk samples were collected weekly during the formal period at a 4:3:3 ratio (morning:afternoon:evening) with 50 mL per collection. Samples were preserved with potassium dichromate and sent to Beijing Dairy Cattle Center for analysis of lactose, milk fat, milk protein percentages, and somatic cell count (SCC) using a MilkoScan 605 multifunctional milk analyzer (Foss Electric, Hillerod, Denmark).

1.4.5 Fecal Sample Collection and Nutrient Apparent Digestibility A digestion trial was conducted on days 54-57 of the formal period. Fecal samples were collected rectally 12 times: at 08:00, 14:00, and 20:00 on day 54; at 02:00, 10:00, 16:00, and 22:00 on day 55; at 04:00, 12:00, 18:00, and 24:00 on day 56; and at 06:00 on day 57. After collection, fecal samples from each cow were pooled and reduced to two 500 g subsamples. One subsample was immediately fixed with 10% hydrochloric acid for CP determination. After the digestion trial, three-day samples from each cow were combined, reduced, dried at 65°C to constant weight, air-equilibrated, ground, and sieved through a 40-mesh screen to obtain air-dried samples for nutrient apparent digestibility determination. Apparent digestibility of DM, CP, NDF, ADF, and OM was determined using the acid-insoluble ash (AIA) method as an internal marker [16].

The apparent digestibility of a nutrient was calculated as:
Apparent digestibility (%) = 100[1-(A×D)/(B×C)]

where: A = AIA content in diet (g/kg); B = nutrient content in diet (g/kg); C = AIA content in feces (g/kg); D = nutrient content in feces (g/kg).

1.5 Statistical Analysis

Experimental data were analyzed using the PROC MIXED procedure of SAS 9.2 software. Linear and quadratic relationships between enzyme inclusion level and response variables were examined. Results are presented as least squares means. Differences were considered significant at $P < 0.05$ and highly significant at $P < 0.01$.

Results

2.1 Effects of Compound Enzyme Preparation on Rumen Fermentation

As shown in Table 3, dietary supplementation with 0.15% compound enzyme preparation significantly increased total volatile fatty acid (TVFA) content compared with the control group ($P < 0.05$). Enzyme addition tended to decrease rumen pH ($P = 0.09$) and increase acetic acid content ($P = 0.08$). No significant effects were observed on propionic acid, butyric acid, valeric acid, isobutyric acid, isovaleric acid, ammonia nitrogen, or microbial crude protein contents ($P > 0.05$), although MCP content showed a linear increasing trend ($P = 0.05$).

2.2 Effects of Compound Enzyme Preparation on Nutrient Apparent Digestibility

Table 4 shows that compound enzyme supplementation significantly improved apparent digestibility of DM and NDF ($P < 0.05$). With increasing enzyme dosage, apparent digestibility of DM, OM, and NDF increased linearly ($P < 0.05$), though no significant differences were observed among treatment groups ($P > 0.05$). While CP and ADF apparent digestibility were not significantly affected ($P > 0.05$), CP digestibility showed a linear increasing trend ($P = 0.05$).

2.3 Effects of Compound Enzyme Preparation on Serum Biochemical Indices

As presented in Table 5, enzyme supplementation tended to increase serum total protein content ($P = 0.09$) and decrease urea nitrogen content ($P = 0.07$). With increasing enzyme dosage, serum TP content increased linearly ($P < 0.05$) while UN content decreased linearly ($P < 0.05$), and creatinine content showed a linear increasing trend ($P = 0.09$).

2.4 Effects of Compound Enzyme Preparation on Performance

Figure 1 [Figure 1: see original paper] illustrates the change trend of 3.5% fat-corrected milk yield during the 8-week experimental period. Throughout the trial, 3.5% fat-corrected milk yield in all treatment groups was higher than in the control group, with the 0.15% group showing the most stable trend.

Table 6 demonstrates that compared with the control group, the 0.10% and 0.15% groups significantly increased 3.5% fat-corrected milk yield by 3.88 and 4.27 kg/d, respectively ($P < 0.05$), and improved feed conversion ratio by 6.86% and 8.00% ($P < 0.05$). The 0.15% group increased milk fat percentage by 12.70% ($P < 0.05$). With increasing enzyme dosage, FCR, 3.5% fat-corrected milk yield, and milk fat percentage showed quadratic changes ($P < 0.05$).

Discussion

3.1 Effects of Compound Enzyme Preparation on Rumen Fermentation

Volatile fatty acids (VFA) are the primary products of carbohydrate fermentation in the rumen and serve as the main source of carbon skeletons and essential energy for growth, reproduction, and lactation, providing 70-80% of the energy required by lactating cows. Therefore, VFA content changes can reflect rumen fermentation patterns and nutrient utilization efficiency in ruminants. Previous studies have shown that exogenous cellulase supplementation at 0.1% significantly increased rumen VFA content in dairy cows, and that cellulase addition to high- or medium-forage diets resulted in higher VFA content than in high-concentrate or control diets. Research on Hu sheep demonstrated that 20 mg/kg exogenous enzyme supplementation optimally increased rumen VFA content by 37% compared with the control. However, other studies reported that fibrolytic enzyme addition increased TVFA content while decreasing the acetate-to-propionate ratio. These findings indicate that enzyme effects on VFA production depend on enzyme type, dosage, dietary concentrate-to-forage ratio, and animal species. In the current study, 0.15% compound enzyme supplementation significantly affected TVFA content and tended to increase acetic acid content, consistent with previous research.

Ammonia nitrogen (NH₃-N) is the primary substrate for microbial crude protein synthesis and reflects the balance between protein degradation and synthesis under specific dietary conditions. NH₃-N and MCP contents also indicate the degree of rumen microbial utilization of dietary nutrients. Enzyme supplementation is thought to promote rumen fermentation by increasing rumen microbial populations. Studies have demonstrated that exogenous fibrolytic enzymes significantly increased rumen microbial populations, including *Ruminococcus flavefaciens*, *Ruminococcus albus*, *Butyrivibrio fibrisolvens*, *Fibrobacter succinogenes*, and *Eubacterium ruminantium*. Other research found that cellulase and

xylanase supplementation improved NDF digestibility and increased MCP production, consistent with our results.

Rumen pH is a comprehensive indicator of rumen fermentation, with optimal pH ranges of 6.2-6.6 for VFA formation and 5.7-6.2 for NH₃-N utilization. In this experiment, rumen pH tended to decrease, likely due to enhanced TVFA production from enzyme supplementation, though all values remained within the normal range.

3.2 Effects of Compound Enzyme Preparation on Nutrient Apparent Digestibility

Research indicates that compound enzyme preparations can increase activities of fibrolytic enzymes, xylanase, and mannanase in both the rumen and small intestine, thereby facilitating nutrient utilization. Studies have reported that commercial compound enzyme preparations promoted DM and CP digestion in dairy cows and improved NDF and ADF digestibility. Other research demonstrated that enzyme preparations primarily containing cellulase and phytase reduced fecal excretion of DM, NDF, ADF, nitrogen, and phosphorus, thus improving nutrient digestibility. However, some studies found no effects of cellulase and xylanase on DM, OM, NDF, and ADF degradation rates. In the present study, compound enzyme supplementation significantly improved DM and NDF apparent digestibility, with linear increases in DM, OM, and NDF digestibility as dosage increased. This may be attributed to the enzyme preparation's ability to adhere to feed, disrupt plant fiber structures, release more reducing sugars, and enhance proliferation of xylanolytic and cellulolytic bacteria, ultimately improving feed utilization and rumen nutrient digestion.

3.3 Effects of Compound Enzyme Preparation on DMI and FCR

Improving dry matter intake (DMI) and feed conversion ratio (FCR) is crucial for enhancing animal performance and reducing feeding costs. Enzyme preparations have been reported to reduce digesta viscosity and increase feed intake. If enzymes exhibit similar viscosity-reducing effects in ruminants, they could increase rumen passage rate, reduce satiety, and promote feed intake. However, increased passage rate might reduce enzyme residence time in the rumen, limiting fiber degradation before enzymes are inactivated by pepsin and low pH in the abomasum. Therefore, enzyme supplementation levels must be controlled within appropriate ranges. In this experiment, 0.10% and 0.15% enzyme supplementation significantly improved FCR and tended to increase DMI, indicating that compound enzyme preparation can promote feed intake to some extent.

3.4 Effects of Compound Enzyme Preparation on Serum Biochemical Indices

Blood albumin (ALB), total protein (TP), urea nitrogen (UN), and creatinine (CR) collectively reflect protein metabolism status. TP consists primarily of

ALB and globulins, with ALB maintaining plasma osmotic pressure. Normal ALB concentration in dairy cows ranges from 29.0-36.6 g/L, and prolonged deficiency indicates protein deficiency. Blood UN originates from dietary and body protein catabolism; increased UN indicates enhanced protein utilization capacity, while decreased UN suggests reduced protein breakdown. Thus, UN changes reflect dietary protein utilization efficiency. CR content indicates protein catabolism and hepatic nitrogen cycling, with increased CR representing enhanced protein catabolism. Previous studies reported that cellulase supplementation significantly increased blood TP and CR while decreasing UN, suggesting that enzymes affect protein metabolism-related blood indices. In the current study, enzyme supplementation tended to increase TP and decrease UN, though not significantly, possibly due to cows' strong homeostatic regulation capacity.

Total cholesterol (TC) and γ -hydroxybutyric acid (BHBA) are lipid metabolites reflecting fat metabolism status. Research has shown that enzyme and phytosterol complexes decreased blood TC and triglyceride concentrations, while other studies found no effects of exogenous cellulase on TC or BHBA, consistent with our results.

Blood glucose (GLU) primarily originates from hepatic glycogenolysis and propionate produced by rumen microbial carbohydrate fermentation, serving as the main energy source and reflecting carbohydrate digestion and absorption capacity. Normal GLU concentration in dairy cows is (3.10 ± 0.47) mmol/L. In this experiment, compound enzyme supplementation did not significantly affect serum GLU content, though numerical increases were observed, consistent with previous findings.

3.5 Effects of Compound Enzyme Preparation on Performance

Milk yield directly affects dairy production profitability, and optimizing enzyme supplementation to increase milk production is a current research focus. Studies have demonstrated that compound enzyme preparations containing cellulase, xylanase, and glucanase significantly increased milk yield at supplementation rates of 10 and 20 g/head/day. Other research showed that 0.1% and 0.2% enzyme supplementation increased milk yield by 0.9 and 1.9 kg/d and 4% fat-corrected milk yield by 0.5 and 2.2 kg/d, with improvements increasing with dosage. However, some studies reported no significant effects on milk yield. In this experiment, 0.10% and 0.15% enzyme supplementation significantly increased 3.5% fat-corrected milk yield compared with the control, with milk yield also showing an increasing trend. This may be attributed to enhanced rumen fermentation, improved nutrient digestibility, and increased FCR. The quadratic relationship between enzyme dosage and 3.5% fat-corrected milk yield suggests a dose-dependent effect.

Milk fat, protein, and lactose contents are key indicators of milk quality. Studies have shown that compound enzyme supplementation improved milk quality,

with liquid enzyme preparations at 1.22 and 3.67 mL/kg significantly increasing milk protein percentage by 1.82% and 2.13%, respectively. Other research demonstrated significant improvements in milk fat and protein percentages, while some studies found no effects. In this experiment, 0.10% and 0.15% enzyme supplementation significantly increased milk fat percentage by 7.30% and 12.70%, respectively. This may be due to enhanced activity of acetogenic and cellulolytic bacteria, increasing VFA and acetic acid production, which serves as an important precursor for milk fat synthesis. The quadratic relationship between enzyme dosage and milk fat percentage may reflect a quadratic relationship between enzyme level and microbial population, with excessive enzyme amounts potentially having negative effects.

Conclusion

1. Dietary supplementation with compound enzyme preparation improved rumen TVFA content and apparent digestibility of DM, OM, and NDF in dairy cows.
 2. Compound enzyme supplementation enhanced feed conversion ratio, 3.5% fat-corrected milk yield, and milk fat percentage in a dose-dependent manner, with 0.15% being the optimal inclusion level.
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References

- [1] He Lirong, Li Aihua. Research and application progress of enzyme preparations in dairy cow diets[J]. *Agricultural Science Research*, 2010, 31(3): 59-64.
- [2] Zhou Bo, Feng Jie. Effects of enzyme preparation and lactic acid bacteria on performance and nutrient digestibility of finishing pigs[J]. *Feed and Animal Husbandry*, 2011(2): 38-40.
- [3] Zhao Peng, Tang Defu, Ru Yingjun, et al. Effects of enzyme supplementation in low-wheat diets on performance and nutrient utilization of broilers[J]. *Journal of Gansu Agricultural University*, 2015, 50(1): 25-30.
- [4] Morgavi DP, Newbold CJ, Beaver DE, 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.
- [5] Lin Jing, Zhao Xinyuan, Du Wen, et al. Effects of compound enzyme preparation on rumen fermentation, nutrient apparent digestibility, and performance of lactating cows[J]. *Chinese Journal of Animal Nutrition*, 2017, 29(6): 2124-2133.
- [6] Gado HM, Salem AZM, Robinson PH, et al. Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation, as well as milk production and composition in dairy cows[J]. *Animal Feed Science and Technology*, 2009, 154(1/2): 36-46.

- [7] Han Zhaoyu, Duan Zhiyong, Ding Liren, et al. Effects of enzyme preparation on milk yield and quality of dairy cows[J]. *Cereal and Feed Industry*, 2008(8): 39-40.
- [8] Peters A, Lebzien P, Meyer U, et al. Effect of exogenous fibrolytic enzymes on ruminal fermentation and nutrient digestion in dairy cows[J]. *Archives of Animal Nutrition*, 2010, 64(3): 221-237.
- [9] NRC. *Nutrient Requirements of Dairy Cattle*[S]. 7th ed. Washington, D.C.: National Academy Press, 2001.
- [10] State Bureau of Quality and Technical Supervision. GB/T 6432-1994 Method for determination of crude protein in feed[S]. Beijing: China Standards Press, 1994.
- [11] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of China. GB/T 20806-2006 Determination of neutral detergent fiber (NDF) in feed[S]. Beijing: China Standards Press, 2007.
- [12] Ministry of Agriculture of the People's Republic of China. NY/T 1459-2007 Determination of acid detergent fiber in feed[S]. Beijing: China Agriculture Press, 2008.
- [13] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China, Standardization Administration of China. GB/T 6438-2007 Determination of crude ash in feed[S]. Beijing: China Standards Press, 2007.
- [14] Wang Jiaqi. *Research Methods in Ruminant Nutrition*[M]. Beijing: Modern Education Press, 2011.
- [15] Makkar HP, Becker K. Purine quantification in digesta from ruminants by spectrophotometric and HPLC methods[J]. *British Journal of Nutrition*, 1999, 81(2): 107-112.
- [16] Keulen JV, Young BA. Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies[J]. *Journal of Animal Science*, 1977, 44(2): 282-287.
- [17] Jia Xianbao. Study on effects of exogenous fibrolytic enzymes on rumen fermentation, digestion, metabolism, and performance of dairy cows[D]. Master's thesis. Hohhot: Inner Mongolia Agricultural University, 2009.
- [18] Li Chaoyun. Effects of chitosan and cellulase on rumen fermentation, methane production, and microbial flora[D]. Doctoral thesis. Yangling: Northwest A&F University, 2014.
- [19] Chen Yu. Effects of exogenous enzymes on rumen fermentation of Hu sheep[D]. Master's thesis. Fuzhou: Fujian Agriculture and Forestry University, 2016.
- [20] Arriola KG, Kim SC, Staples CR, et al. Effect of fibrolytic enzyme application to low- and high-concentrate diets on the performance of lactating dairy cattle[J]. *Journal of Dairy Science*, 2011, 94(2): 832-841.
- [21] Beauchemin KA, Colombatto DC, Morgavi DP, et al. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants[J]. *Journal of Animal Science*, 2003, 81(14 Suppl. 2): 1061-1074.
- [22] Huhe, Jin Hai, Xue Shuyuan, et al. Study on effects of exogenous fibrolytic

- enzymes on rumen fiber-degrading bacteria in dairy cows[J]. *Animal Husbandry and Feed Science*, 2011, 32(5): 25-28.
- [23] Yang WZ, Beauchemin KA, Rode LM. 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.
- [24] Hristov AN, McAllister TA, Cheng KJ. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility[J]. *Journal of Animal Science*, 1998, 76(12): 3146-3156.
- [25] Hristov AN, McAllister TA, Cheng KJ. Stability of exogenous polysaccharide-degrading enzymes in the rumen[J]. *Animal Science and Technology*, 1998, 76(1/2): 161-168.
- [26] Xu Xuewen, Liu Zhengya, Tian Wen, et al. Effects of different compound enzyme preparations on performance and nutrient digestibility of dairy cows[J]. *Cereal and Feed Industry*, 2012, 12(11): 51-54.
- [27] Knowlton KF, Taylor MS, Hill SR, et al. Manure nutrient excretion by lactating cows fed exogenous phytase and cellulase[J]. *Journal of Dairy Science*, 2007, 90(9): 4356-4360.
- [28] Xie Xiangxue, Du Hongfang, Chen Shuqin, et al. Application and prospect of exogenous enzyme preparations in ruminants[J]. *Chinese Journal of Animal Nutrition*, 2016, 28(4): 1011-1019.
- [29] Choct M. Enzymes for the feed industry: past, present and future[J]. *World's Poultry Science Journal*, 2006, 62(1): 5-16.
- [30] Yang LL, Lv YY, Sun GQ. Effect of brewer's grains on milk production and blood biochemical parameters of dairy cows[J]. *Chinese Animal Husbandry and Veterinary Medicine*, 2014, 50(13): 51-56.
- [31] Song Ping. Measurement and analysis of blood biochemical indices of dairy cows under different feeding systems[D]. Master's thesis. Harbin: Northeast Agricultural University, 2007.
- [32] Hu Tianqin. Effects of different levels of enzyme preparation and phytosterol complex on performance and blood indices of dairy cows[D]. Master's thesis. Nanjing: Nanjing Agricultural University, 2013.
- [33] Lv Chao. Effects of roughage combinations on nitrogen and phosphorus excretion and farmland carrying capacity of dairy cows[D]. Master's thesis. Zhengzhou: Henan Agricultural University, 2012.
- [34] Silva TH, Takiya CS, Vendramini THA, et al. Effects of dietary fibrolytic enzymes on chewing time, ruminal fermentation, and performance of mid-lactating dairy cows[J]. *Animal Feed Science and Technology*, 2016, 221: 35-43.
- [35] Sutton JD, Phipps RH, Beever DE, et al. Effect of method of application of a fibrolytic enzyme product on digestive processes and milk production in Holstein-Friesian cows[J]. *Journal of Dairy Science*, 2003, 86(2): 546-556.
- [36] Yang Zekun. Effects of ruminant-specific compound enzyme preparation on rumen fermentation, blood indices, and performance of dairy cows[D]. Master's thesis. Yangling: Northwest A&F University, 2017.
- [37] Nsereko VL, Beauchemin KA, Morgavi DP, 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.

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