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Effects of fibrolytic and amylolytic compound enzyme preparation on rumen fermentation, serum parameters and production performance in primiparous early-lactation dairy cows

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Research Article

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Abstract

This research communication reports the effects of a compound enzyme preparation consisting of fibrolytic (cellulase 3500 CU/g, xylanase 2000 XU/g, β-glucanase 17 500 GU/g) and amylolytic (amylase 37 000 AU/g) enzymes on nutrient intake, rumen fermentation, serum parameters and production performance in primiparous early-lactation (47 ± 2 d) dairy cows. Twenty Holstein–Friesian cows in similar body condition scores were randomly divided into control (CON, $n = 10$) and experimental (EXP, $n = 10$) groups in a completely randomized single-factor design. CON was fed a basal total mixed ration diet and EXP was dietary supplemented with compound enzyme preparation at 70 g/cow/d. The experiment lasted 4 weeks, with 3 weeks for adaptation and then 1 week for measurement. Enzyme supplementation significantly increased diet non-fibrous carbohydrates (NFC) content as well as dry matter intake (DMI) and NFC intake ($P < 0.05$). EXP had increased ruminal butyrate and isobutyrate percentages ($P < 0.01$) but decreased propionate and valerate percentages ($P < 0.05$), as well as increased serum alkaline phosphatase activity and albumin concentration ($P \leq 0.01$). Additionally, EXP had increased milk yield (0.97 kg/d), 4% fat corrected milk yield and energy corrected milk yield, as well as milk fat and protein yield ($P < 0.01$). In conclusion, dietary supplementation with a fibrolytic and amylolytic compound enzyme preparation increased diet NFC content, DMI and NFC intake, affected rumen fermentation by increasing butyrate proportion at the expense of propionate, and enhanced milk performance in primiparous early-lactation dairy cows.

Supplementing exogenous enzymes is a safe biological method to promote animal performance and the production of enzymes is becoming cheaper and more efficient (Zilio *et al*., 2019). Cellulose and xylanase are the most frequently investigated fibrolytic enzymes in dairy cattle (Zilio *et al*., 2019) and show different effects on nutrient utilization (Yang *et al*., 2000) and production performance (Murad and Azzaz, 2010). Two meta-analyses (Arriola *et al*., 2017; Tirado-González *et al*., 2018) demonstrated positive overall effects for fiber digestion and milk production (Adesogan *et al*., 2019). Dietary supplementation of amylolytic enzymes to cows promoted rumen fermentation (Noziere *et al*., 2014), milk yield (Tricarico *et al*., 2005) and feed efficiency (Andreazzi *et al*., 2018) without leading to acidosis. However, different results also exist (Andreazzi *et al*., 2018) so it is fair to say that consensus has not been reached.

In theory, exogenous fibrolytic and amylolytic enzymes are considered to perform synergistic effects when supplementing in combination (Tricarico *et al*., 2008). Nevertheless, as far as we know, only two studies reported on this (Hristov *et al*., 2008; Zilio *et al*., 2019). No effect was found on nutrient ingestion and digestion, rumen fermentation, or milk performance. This might be attributed to the differences in delivery method (Beauchemin *et al*., 2003; Hristov *et al*., 2008), the time of delivery and the proportion of the diet delivered at each time (Adesogan, 2005; Zilio *et al*., 2019). To control the variation, we added the enzyme preparation at the moment of total mixed ration (TMR) production and offered twice per day (Adesogan, 2005; Adesogan *et al*., 2019). Moreover, the variation in results probably is attributed to the lactation stage (Beauchemin *et al*., 2003) and parity (Wathes *et al*., 2007) of cows, which was different between Hristov *et al*. (2008: late-lactation multiparous) and Zilio *et al*. (2019: mid-lactation multiparous). We used early-lactation primiparous

cows since they seem to benefit more from the enzyme supplementation (Bachmann *et al.*, 2018).

Given these various observations, we hypothesized that dietary supplementation with a compound enzyme preparation of fibrolytic (cellulase, xylanase, and β-glucanase) and amylolytic (amylase) enzymes to primiparous early-lactation dairy cows would boost nutrient intake, promote rumen fermentation as well as energy metabolism and enhance milk production in cows.

Materials and methods

The experiment was conducted at the Modern Farm (Baoji, China) and the Laboratory of Animal Nutrition at Northwest A&F University (Yangling, Shaanxi, China). All experimental procedures were approved by the Northwest A&F University Animal Care and Use Committee.

Twenty primiparous early-lactation (47 ± 2 d in milk) Holstein cows of similar body condition score were randomly divided into control (CON, $n = 10$) and experimental (EXP, $n = 10$) groups as a completely randomized single-factor design. The CON was only fed a basal TMR diet and the EXP was dietary supplemented with compound enzyme preparation at 70 g/cow/d. Enzyme preparation was added to TMR during its production twice daily. The experiment lasted 4 weeks, with 3 weeks of adaptation followed by a 1-week experimental period. Cows were fed twice per day (0600 and 1400) with at least 5% residues in the feed trough, given free access to water and were milked three times daily (0000–0100, 0700–0800, and 1400–1500).

The basal TMR diet (online Supplementary Table S1) was prepared twice daily before feeding. The compound enzyme preparation (Guangdong VTR Bio-Tech Co., Ltd.) contained fibrolytic enzymes (cellulase 3500 CU/g, xylanase 2000 XU/g, and β-glucanase 17 500 GU/g) and amylolytic enzyme (amylase 37 000 AU/g) (enzyme activities determination details are shown in the online Supplementary File). Feed intake and milk production were recorded daily, whilst the chemical composition and particle-size distribution of feed samples, as well as the physical and chemical indices and SCC of milk samples, were measured for three consecutive days (the first to third day of experimental period). Due to limited labor availability, the rumen pH and volatile acid profile, as well as the serum parameters were sampled for one day. Further experimental details are provided in the online Supplementary File.

Statistical analysis

The study was performed using a completely randomized single-factor design. The daily averages of feed intake, production and feed analysis data were calculated and used for further statistical analysis. For statistical analyses, SPSS software (Version 22.0, SPSS Inc., Chicago, USA) was used to determine the differences of all measures between control and experimental groups, with supplementation of compound enzyme preparation as the fixed factor and the cow as a random factor. The model employed was:

$$Yij = \mu + \text{treatment } i + \text{cow } j + \varepsilon ij$$

where Yij = the kth observation of the jth cow in the ith treatment, μ = the overall mean, treatment i = the fixed effect of the ith treatment ($i = 0$ to 1), cow$j$ = the random effect of the jth cow ($j = 1$ to 10), ϵij = the residual error associated with the jth cow in the ith treatment. All results were expressed as the mean and SEM Significance was declared at $P \leq 0.05$.

Results and discussion

Supplementing compound enzyme preparation increased diet non-fibrous carbohydrate content (NFC) ($P < 0.05$: online Supplementary Table S2). This could probably be explained by the pre-digestive mechanism of fibrolytic enzymes (Adesogan *et al.*, 2019), such that the fibrolytic enzymes could partially solubilize acid and neutral detergent fibers, releasing sugars and free or monomeric hydroxycinnamic acids before feeding (Romero *et al.*, 2015). Moreover, EXP had improved dry matter intake (DMI) and NFC intake ($P < 0.05$: Supplementary Table S2). The improvement of DMI is consistent with studies of orally supplied fibrolytic enzymes (Beauchemin *et al.*, 2003; Adesogan, 2005). This is probably due to the improved palatability of the increased sugars released from the hydrolyzation of fiber (Adesogan, 2005), and the reduced rumen and gut fill by the improved digestion rate (Beauchemin *et al.*, 2003, Adesogan, 2005).

EXP had increased molar percentage of rumen butyrate and isobutyrate ($P < 0.01$) as well as decreased molar percentages of propionate and valerate ($P < 0.05$: Table 1). These results are similar to the research of Tricarico *et al.* (2005), who found improved acetate and butyrate as well as reduced propionate molar proportions in steers and lactating dairy cows with dietary addition of α-amylase. In a subsequent study, Tricarico *et al.* (2008) hypothesized a cross-feeding mechanism that the supplemented α-amylase and fibrolytic enzymes would hydrolyze amylose, cellulose and xylans into oligosaccharides, thereby providing substrate to non-fibrolytic or non-amylolytic bacteria, giving these bacteria a competitive advantage. This hypothesis could probably explain the current study. Due to the ratio between fibrolytic and amylolytic enzymes, or the ratio of forage to starch in diet, or the presence of butyrate producing bacteria (*Selenomonas ruminantium* GA192) that could use both malto- and xylo-oligosaccharides (Tricarico *et al.*, 2008) as substrate, the supplemented enzymes in the current study provided a competitive advantage to butyrate producing bacteria. EXP exhibited an increase in serum alkaline phosphatase (ALP) activity ($P < 0.01$) and albumin concentration ($P < 0.05$: Supplementary Table S3). The serum ALP activity and albumin concentration of both CON and EXP were in the normal and healthy range (70–144 U/l and and 27–47 g/l, respectively) as reported by Cozzi *et al.* (2011) and Lager and Jordan (2012).

EXP exhibited increased yield of milk, fat corrected milk (FCM), energy corrected milk (ECM), milk fat and milk protein (all $P < 0.01$ or better: Table 1). Improved milk performance was also shown in the studies of supplemented fibrolytic enzymes (Arriola *et al.*, 2017) and amylolytic enzymes (Tricarico *et al.*, 2005; Andreazzi *et al.*, 2018; Bachmann *et al.*, 2018). It was attributed to the improvement of DM and NDF digestibility by Arriola *et al.* (2017), and to the promotion of rumen starch fermentation and the propionate absorption for liver gluconeogenesis by Andreazzi *et al.* (2018). By contrast, in the research of Tricarico *et al.* (2005), the nutrient digestibility was not affected but the rumen VFA profile (increased butyrate and decreased propionate proportions) and serum metabolite concentration (higher BHBA, NEFA concentrations and unaffected glucose concentration) were changed, indicating that the supplementation of amylolytic enzyme might improve milk yield by affecting ruminal fermentation and concomitantly changing serum metabolite concentrations. Similarly, the enhanced milk performance in the current

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Table 1. Effects of compound enzyme preparation on rumen fermentation (n = 10 cows/group) and production performance in dairy cows (n = 3, milk samples were collected from the 1st to 3rd day of the experimental period)

Item	CON[1]	EXP[2]	SEM	P-value
Rumen fermentation				
pH	6.56	6.49	0.063	0.18
Total volatile fatty acid (mmol/l)	114.22	104.89	5.071	0.37
Acetate (%)	36.51	38.17	0.574	0.16
Propionate (%)	40.01[a]	36.36[b]	0.863	0.03
Butyrate (%)	16.21[b]	18.55[a]	0.432	<0.01
Valerate (%)	3.81[a]	3.23[b]	0.138	0.03
Isobutyrate (%)	0.87[b]	1.10[a]	0.041	<0.01
Isovalerate (%)	2.84	2.85	0.122	0.97
Acetate: Propionate	0.93	1.07	0.04	0.07
Milk yield (kg/d)				
Actual milk yield	37.40[b]	38.37[a]	0.138	<0.01
FCM	32.89[b]	34.01[a]	0.254	<0.01
ECM	36.23[b]	37.50[a]	0.29	<0.01
Fat	1.17[b]	1.23[a]	0.013	<0.01
Lactose	2.03	2.04	0.005	0.09
Protein	1.24[b]	1.29[a]	0.011	<0.01
Milk composition (%)				
Fat	3.05	3.16	0.140	0.70
Lactose	5.28	5.24	0.023	0.44
Protein	3.23	3.31	0.033	0.27
Total solids	11.69	11.84	0.145	0.60
Urea-Nitrogen (mg/100 ml)	14.42	13.67	0.607	0.54
SCC (10^3/ml)	46.12	52.44	7.551	0.68
Feed efficiency[3]	1.59	1.55	0.036	0.65

[1]Control (CON) group, without supplementation of compound enzyme preparation.
[2]Experimental (EXP) group, with supplementation of compound enzyme preparation.
[3]Feed efficiency = FCM yield (kg/d)/DMI (kg/d).
[a,b]Different superscripts within a row indicate a significant difference (P < 0.05). SEM, standard error of the mean; FCM, 4% Fat corrected milk yield = Actual milk yield (kg/d) × (0.4 + 15 × Milk fat content (%)); ECM, Energy corrected milk yield = Actual milk yield (kg/d) × (0.3246 + 12.86 × Milk fat content (%) + 7.04 × Milk protein content (%)); SSC, somatic cell count.

study is probably because of the effect on ruminal fermentation. However, it should be remembered that increased butyrate and decreased propionate proportion is normally considered to reduce blood glucose concentration because propionate is gluconeogenic and butyrate is ketogenic, and butyrate has been reported to inhibit the hepatic uptake of propionate. Tricarico *et al.* (2005) found that when cows were supplemented with α-amylase with a dose of 240 DU per kg TMR, the increase in rumen butyrate, which was at the expense of propionate, was not large enough to decrease liver gluconeogenesis or blood glucose content. Additionally, the rumen butyrate concentration has been reported to have a strong positive correlation with milk yield (Seymour *et al.*, 2005).

Recall that the two previous studies using combined fibrolytic and amylolytic enzymes (Hristov *et al.*, 2008; Zilio *et al.*, 2019) did not find any effect on milk yield. One explanation for Hristov *et al.* (2008) is insufficient enzyme dose. Another explanation is the delivery method of direct supplementation into rumen (Beauchemin *et al.*, 2003). Because the pre-ingestive effects of enzymes on feed were lacking, and the homogeneity between feed and enzymes was decreased, the lack of effect on milk yield for Zilio *et al.* (2019) could probably be attributed to the differences in time and diet portion of adding enzyme. In the research of Adesogan (2005), the enzymes were supplemented once a week into concentrate during its preparation. This delay may have been too long, resulting in reduced enzyme activity. These workers did show positive responses on production when utilizing high concentrate to forage ratio (62:38) in the diet, but not when a lower concentrate to forage ratios (45:55 to 40:60) was used Adesogan, 2005). Hence, a proper diet composition for enzyme addition should consider the concentrate to forage ratio. The concentrate to forage ratio of Zilio *et al.* (2019) was about 52:48, which is closer to the range of 45:55 to 40:60. Therefore, adding enzymes into the concentrate may not be effective.

Lactation stage (Beauchemin *et al.*, 2003) and parity (Wathes *et al.*, 2007) of cows might result in variable responses. As indicated by Bachmann *et al.* (2018), the supplementation of exogenous amylase only promoted the milk yield of high-producing (≥32 kg milk/day) early lactation primiparous cows, whilst late-lactation lower producing primiparous cows as well as early- and late-lactation multiparous cows were not affected. Late- and mid-lactation multiparous cows were used in the research of Hristov *et al.* (2008) and Zilio *et al.* (2019). Early-lactation cows are more responsive to exogenous enzyme addition due to higher energy requirements caused by calving and higher milk yields (Beauchemin *et al.*, 2003), and primiparous cows have larger magnitude of response than multiparous, because they have not previously experienced calving and lactation, therefore they are more sensitive to energy in the early lactation period (Wathes *et al.*, 2007).

In conclusion, the dietary supplementation of a fibrolytic (cellulase, xylanase, and β-glucanase) and amylolytic (amylase) compound enzyme preparation increased diet NFC content, DMI and intake of NFC, affected rumen fermentation by increasing butyrate proportion at the expense of propionate, and enhanced milk performance in primiparous early-lactation dairy cows.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0022029924000475

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