# Applications of NSP enzymes in ruminants

## Abstract

The use of exogenous NSP enzyme preparations in ruminant animals is complex and highly variable because of the characteristics of rumen fermentation and inherent enzyme synthesizing in rumens, as well as the complexity of raw feed materials. This review summarized the applications of NSP enzymes in beef cattle and dairy cows based on a meta-analysis and CJ youtell trials.

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## Background

In ruminants, digestion of the structurally complex fibrous compounds in plant cell walls is accomplished through the enzymic action of the ruminal microflora, but it is far from complete. There is considerable room to enhance the utilization of fibrous feeds by ruminant livestock. It is known that exogenous enzymes can increase fiber degradation by facilitating microbial colonization to the insoluble plant cell walls (Colombatto et al., 2003).

Adult ruminant animals have special digestive system including rumen, reticulum, omasum, and abomasum. The rumen is responsible for breaking down the complex plant materials. It contains a diverse group of bacteria, protozoa, and methane -producing archaea that aid in the degradation of plant material.

21 enzymes have been identified to break down plant-based polysaccharide cell walls in the rumen, all of which are produced by normally functioning rumen microorganisms (White et al., 1993). However, the amount of ruminal endogenous enzymes might be insufficient due to the complex structural carbohydrates and lignin from fibrous feeds. Exogenous enzyme preparations for ruminants are marketed primarily based on their capacity to degrade plant cell walls and as such, are often referred to as cellulases and/or xylanases, which are NSP enzymes. The main advantages of NSP enzymes are they can effectively improve the digestibility and absorption rate of feed nutrients, reduce the emission of greenhouse gases, and reduce the pollution of animal manure. In actual, exogenous enzymes in ruminants have already been examined in 1960s (Rust et al., 1965; Burroughs et al., 1960), and their effects have already been approved by many peer-reviewed articles. Meanwhile, there are also some articles reporting exogenous enzymes have no effects and even negative effects (McAllister et al.,2000; Svozil et al., 1989; Theurer et al., 1963). Exogenous enzymes could exert several effects, both on the gastrointestinal microflora and on the ruminant animal itself. It is highly probable, therefore, that physiological responses to exogenous enzymes are multifactorial in origin. In the review, we only talked about the NSP enzymes in ruminants and shared some research by CJ youtell for a better understanding of enzymes used in ruminants.

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## Carbohydrate metabolism in rumen

Rumen is the main place for digesting fibrous substances and the place where microorganisms consume soluble carbohydrates. Starch and fibrous substances are broken down by enzymes and eventually form pyruvate, an intermediate substance, and after a series of biochemical reactions, form volatile fatty acids such as acetic acid, propionic acid, and butyric acid, as well as gases such as methane (Figure 1). In a normal situation, the digestion of starch and non-starch polysaccharides (fibrous substances) is in dynamic equilibrium. However, for high yielding cows, they are always with high feed intake. High feed intake or eutrophication status exceeds the ability of rumen microorganisms to produce enzymes, or high load fermentation activities. Especially diets with high concentrate, high grain, and high starch properties, are particularly prone to acidosis, which affects rumen microbial activity and micro-ecosystem, and cannot ensure the integrity of the rumen fermentation system.

There are three reasons for adding enzyme preparations to ruminants:

- **To assist in solving the problem of ensuring a certain amount of coarse feed digestion;**
- **To maintain and ensure the biological activity and microecological issues of rumen microorganisms;**
- **To solve the problem of acidosis that high-yield cows need due to high energy grain input.**

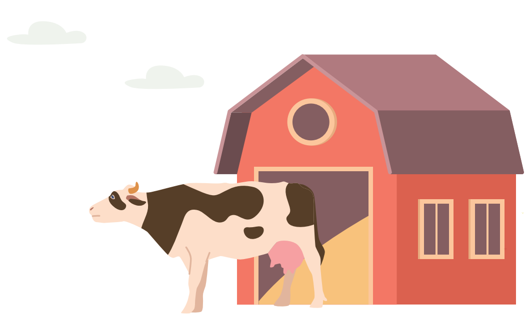
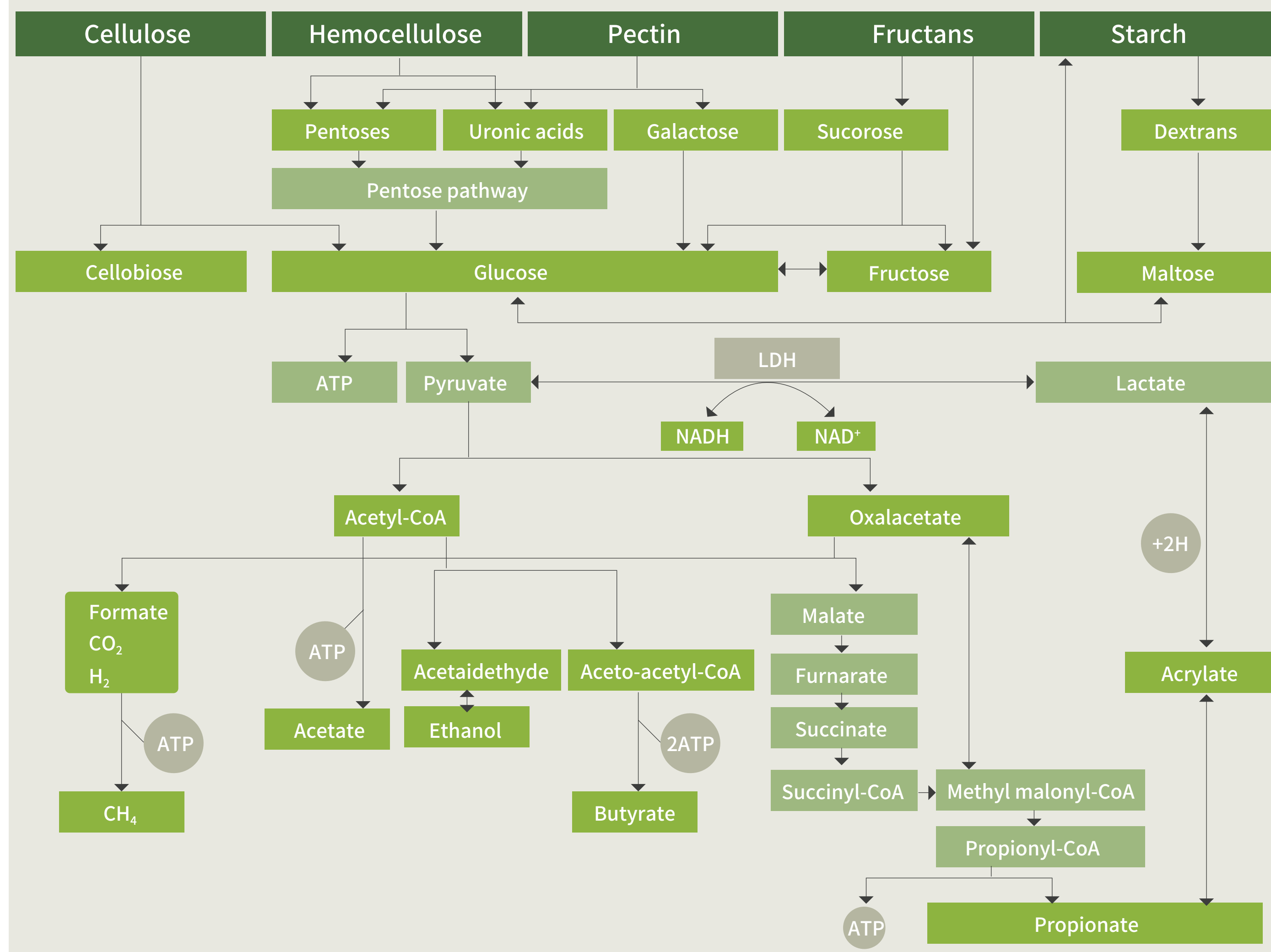


Figure 1. Carbohydrate metabolism in rumen



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## In vitro fermentation to evaluate exogenous enzymes

Comparing with swine and poultry, it's not easy to effectively evaluate the effects of exogenous enzymes in ruminants. Fortunately, with the deepening of research on ruminant enzyme preparation, researchers have built several methods, which are mainly in vitro incubations of enzyme and feed with ruminal contents and measurement of the disappearance of the substrate, pH change, volatile fatty acids (VFAs) production, gas production and so on (McAllister et al., 2001).
Exogenous enzyme preparations can improve the fermentation function of the rumen in vitro. Application of xylanase could improve in vitro gas production fermentation of wheat straw(Togtokhbayar et al., 2015), exogenous enzyme can reduce NDF and increase reducing sugar, cellulase and xylanase activities in the rumen fluid (Wang et al., 2001). CJ youtell conducted a similar in vitro fermentation trial using a mixture of xylanase, cellulase and β-glucanase, and the results are shown in Table 1. The total gas production increased, indicating that rumen belching and ruminating ability probably increased. The microbial protein increased, and the $NH_3$-N decreased in experiment groups which meant exogenous enzyme increased the number of microorganisms and enhanced the utilization of inorganic nitrogen sources.

Table 1. Effects of CJ NSP enzymes in vitro fermentation

| Treatment | Control* | Exp.1 (0.05%) | Exp.2 (0.10%) | Exp.3 (0.20%) | SEM |
|---|---|---|---|---|---|
| Gas production, mL | 182.6 | 192.1 | 194.8 | 231.9 | 14.83 |
| pH | 6.17 | 6.25 | 6.27 | 6.29 | 0.02 |
| T-VFA, mM | 131.7 | 95.7 | 103.5 | 84.5 | 12.02 |
| Acetic acid (A), %T-VFA | 65.6 | 67.5 | 67.4 | 68.3 | 0.90 |
| Propionic acid (P), % T-VFA | 16.4 | 16.9 | 16.2 | 16.3 | 0.63 |
| Butyric acid, % T-VFA | 11.2 | 10.2 | 10.1 | 10.1 | 0.33 |
| A/P# | 4.0 | 4.0 | 4.2 | 4.2 | 0.20 |
| $NH_3$-N, mM | 11.7 | 10.7 | 10.8 | 10.9 | 0.94 |
| Microbial protein, mg/mL | 0.66 | 0.73 | 0.78 | 0.82 | 0.06 |

*Fermented substrates: corn silage 20.9%, alfalfa 18.4%, rye 5.1% corn 35.3%, soybean meal 20.3% ; # A/P means the ration of acetic acid / propionic acid, which reflects the type of fermentation in the rumen. Exp.1/2/3 means three different dosage of 0.05%,0.1% and 0.2%, respectively.

## Effects of exogenous enzymes in beef cattle

According to the results of a meta-analysis of exogenous enzyme in ruminants (Table 2), it i s known that the ADG increased by 31 g/d in cattle fed diets containing <50% grasses and treated with exogenous fibrolytic enzymes (EFE). In low-forage grass-based diets (F:C <50%), EFE enzymes also had positive effects on F/C (5.8 vs 5.6). In a high F/C ration (F:C ≥ 50%), the ADG just increased by 4 g/d and the F/C increased 0.4 (10.1 vs 10.5). The analysis indicates that the effects of exogenous enzymes are probably influenced by the diet types, especially the F/C ratio. Compared to accurately calculating ADG, F:C and like that, we can learn a lot of digestive information from animal feces. In previous research on beef cattle by using CJ NSP enzymes, the experiment analyzed the dry matter (DM) and the proportions of different fiber lengths in feces under two kinds of feeding modes: tie stalls and free stalls by using fecal sieve. The principle of fecal sieve is the same with that of Penn State Particle Separator. Finally, the results indicated that CJ NSP enzymes decreased the DM and promoted the percentage of shorter crude fibers in feces under both feeding modes (Figure 2).

Table 2. Effects of using exogenous fibrolytic enzymes (EFE) on beef cattle productive performance (Tirado-González et al., 2018)

| Variable | N | Forage: Concentrate ratio (F:C) | | | |
|---|---|---|---|---|---|
| | | F:C ratio＜50% | | F:C ratio≥50% | |
| | | Control | EFE | Control | EFE |
| BW(kg) | 45 | 324.1±81.9 | 305.2±63.8 | 219.8±124.8 | 233.3±112.9 |
| DMI(kg/d) | 45 | 8.3 | 8.1 | 8.3 | 9.4 |
| ADG(g/d) | 45 | 1542.9 | 1573.3 | 1124 | 1128 |
| F/C(DMI/ADG) | 45 | 5.8 | 5.6 | 10.1 | 10.5 |

*EFE, exogenous fibrolytic enzymes mainly xylanse and cellulase.

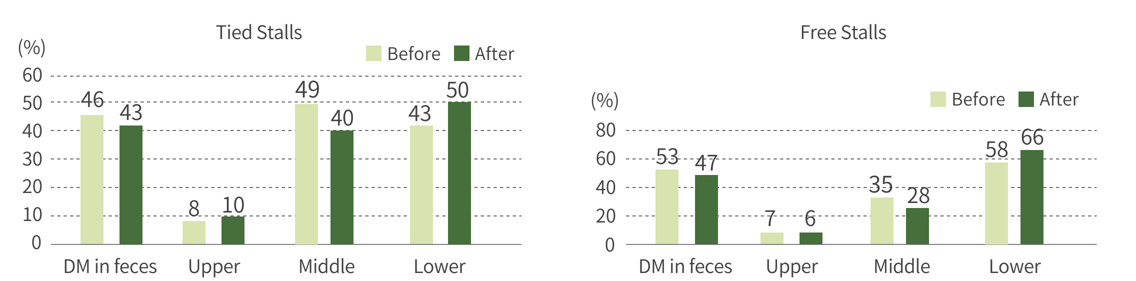


Figure 2. The effects of CJ NSP enzyme on feces dry matter of beef cattle in tied stalls (A) and free stalls (B).

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## Effects of exogenous enzymes in dairy cows

The effects of exogenous enzymes on milk production in dairy cows was first examined in the mid-1990s. However, the effects of exogenous enzymes on milk yield are in contrast sometimes. Mohamed et al. (2013) found that xylanase increased milk production by 1.5 kg/d (3.8%). Another research applying cellulase and xylanase mixture to diets with alfalfa silage, hay and concentrate failed to increase milk yield (Nussio, 1997) in contracts, two similar enzyme preparations in corn silage and concentrate base diets increased milk production by 2.5 kg/d (Kung, 1996). The use of complex degrading enzymes as a supplement for ruminants could increase the digestible energy of high-fiber and forage-based diets and reduce the amount of feed required per unit of milk or live weight (Meale et al., 2014).

According to the results of a meta-analysis on dairy cows (Table 3), NSP enzymes applied to high-forage diets (F:C ratio ≥50%) had positive effects on milk production (33 vs 34.9, +1.9 kg/d), milk protein (1007.5 vs 1106.9, +99.4 g/d) and milk fat (1179.6 vs 1272.6, +83 g/d). The results are opposite in low-forage diets (F:C ratio＜50%). The research on dairy cows also indicates that the effect of enzyme preparations is influenced by the type of diet. On dairy cows, farmers always want to increase milk production, especially in the later stages of milk production. As a trial in last stage of lactation, CJ NSP enzyme increased 1kg, 1kg and 0.8kg of milk yield of dairy cows in the last stage of lactation respectively at 15 days, 30 days and 45 days after feeding enzymes (Figure 3). It's meaningful for improving the economic benefits of cows in the late lactation stage.

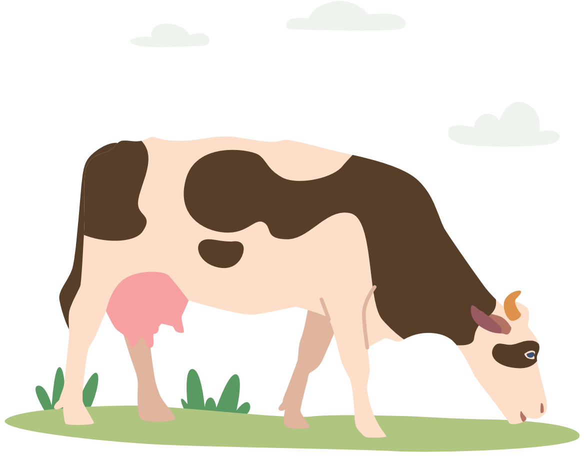


Table 3. Effect of using exogenous fibrolytic enzymes (EFE) in diets with high and low forage on dairy cows production performance (Tirado-González D N et al.,2018)

| Variable | N | Forage: Concentrate ratio (F:C) | | | |
|---|---|---|---|---|---|
| | | F:C ratio＜50% | | F:C ratio≥50% | |
| | | Control | EFE | Control | EFE |
| BW(kg) | 52 | 543.9±31.2 | 548.8±26.5 | 621.7±41.9 | 612.9±47.5 |
| Days in milk production | 52 | 98.7±15.4 | 98.7±15.4 | 72.2±40.4 | 83.4±42.0 |
| DMI (kg/d) | 52 | 20.3 | 20.1 | 21.8 | 21.2 |
| ADG (g/d) | 52 | 0.5 | -0.5 | 1.1 | 1.5 |
| Milk production (kg/d) | 52 | 28.4 | 28.1 | 33 | 34.9 |
| Milk protein (g/d) | 52 | 924.6 | 881.3 | 1007.5 | 1106.9 |
| Milk protein (g/d) | 52 | 1136.6 | 1101.2 | 1179.6 | 1262.6 |

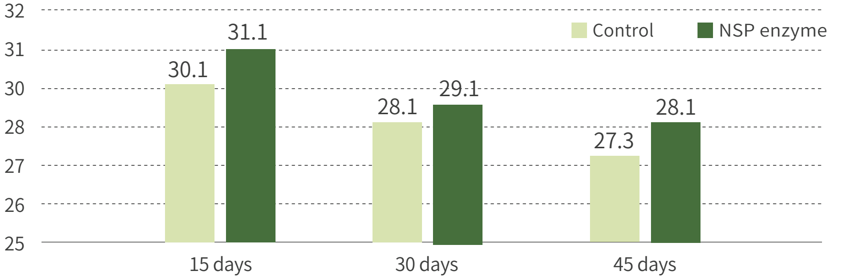



Figure 3. The effects of CJ NSP enzymes on milk yield of dairy cows at last stage of lactation

## Factors that probably influence enzyme effects

### Enzyme types

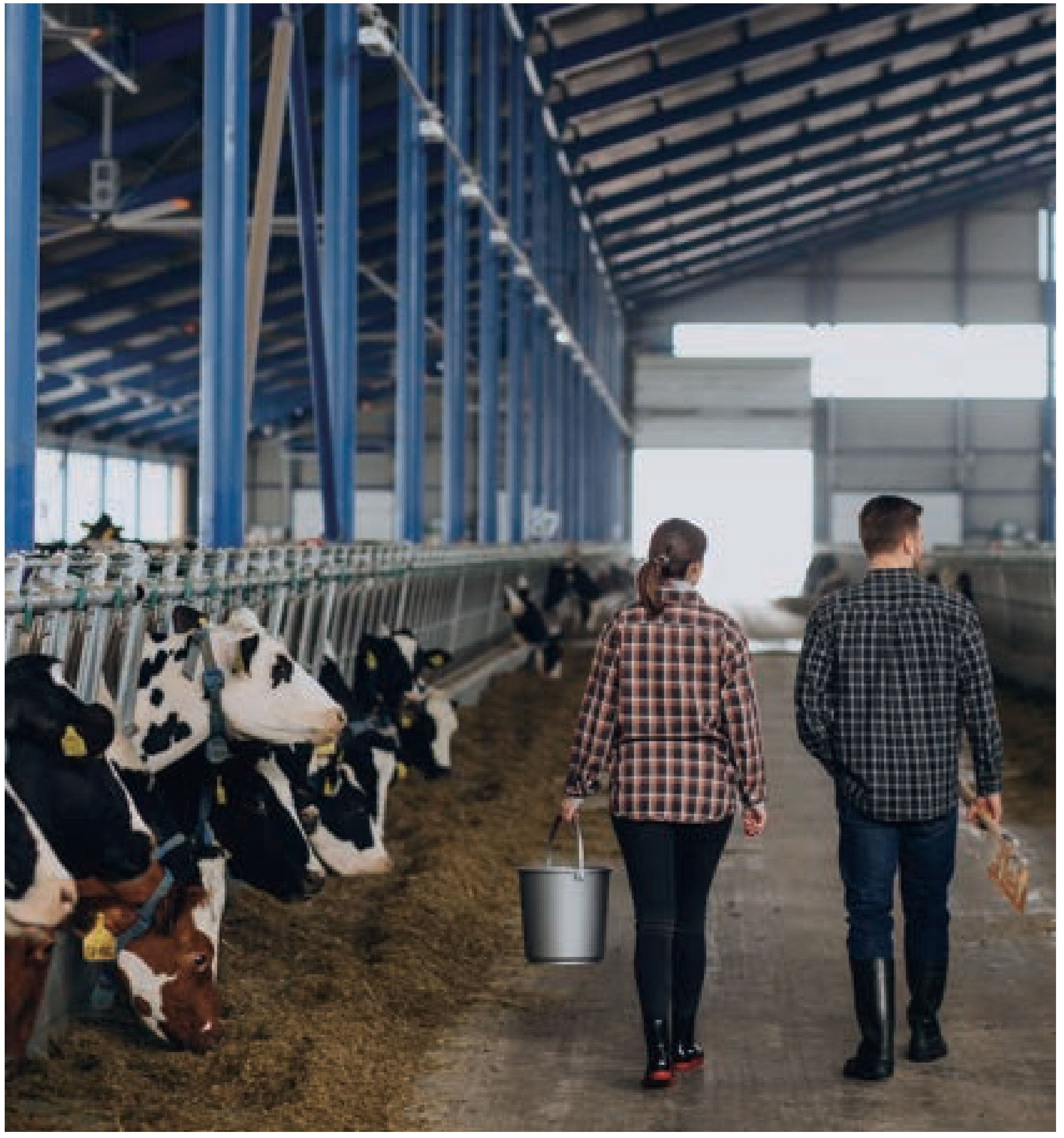
According to the digestive physiology and dietary composition of ruminants, cellulase and xylanase should be mainly selected in the rumen, because they mainly degrade cellulose and hemicellulose into hexose and pentose, and further decompose them into volatile fatty acids (VFAs), which are used by the body. Multi-enzyme cocktails may work better than extracts of almost pure enzymes (Yu et al., 2005), and the proportion of NSP enzymes also had an influence on the effects (Tirado-González et al., 2018).

### Adding modes to use enzymes

Different addition methods can also have a certain impact on the effectiveness of enzyme preparations. Adding enzymes in TMR is currently a common practice for ruminant enzymes. Meanwhile, it also can be added to commercial concentrate feed at a certain dosage. Due to the large amount of coarse feed used by ruminants, enzyme preparations can also be used for pretreatment in the coarse feed, especially the use of cellulase and other NSP enzymes. More commonly, enzyme preparations are used in silage, which is more convenient to operate. Many experiments on ruminant enzymes also use enzyme preparations to treat silage, achieving good results and improving dry matter and NDF digestibility. Ordaz S. (2017) reported that a mixture of cellulase and xylanase had more pronounced effects in the late-maturity alfalfa cut than in the early-maturity which suggested that certain cellulase and xylanase have the potential as a management resource on farms aiming to improve the nutritive value of forages with a high fiber concentration.

### Dose of enzyme used

Optimum doses of fibrolytic enzymes have not been well established now but interestingly, several publications have reported that high levels of enzymes resulted in lower milk yields than moderate levels of enzyme treatment (Beauchemin et al., 1995; Kung et al., 2000; Lewis et al., 1999). Over-treatment of feeds with enzymes may result in blocking sites that may otherwise be available for microbial enzymatic digestion or may prevent attachment by rumen microbes (Jr. Limin Kung, 2006). More research will be needed in this area.



## Conclusion

Non-starch polysaccharide enzymes can improve the crude fiber digestibility and production performance of ruminants, but their effects are closely related to the type of feed, and the physiological period of animals, and the types and dosages of exogenous enzymes.

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