

Research Report:

Effects of Feeding Exogenous Fibrolytic Enzymes to Dairy Cows



The Dairy Knowledge Center (DKC) is a global platform where progressive dairy research and management practices are shared. The DKC's multidisciplinary team researches and brings insights and strategies that help dairy leaders gain competitive advantage on their operations. Innovation is the main core value at DKC. Working with an extensive network of external collaborators, leading scholars, and partners, we explore ideas that will transform the dairy industry of tomorrow.

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Introduction

Dairy cattle and other ruminants are biologically designed to convert forages and other fibrous feeds into high quality products such as meat and milk. Forages are in general the least expensive source of energy for dairy cows. However, the efficiency of converting forages to milk is limited by the digestibility of forage cell walls. Under ideal feeding conditions cell wall digestibility in the total digestive tract is still generally less than 65%⁴². A recent study published by Danish researchers in the *Animal Feed Science and Technology* journal²⁸ investigated the importance of corn silage fiber digestibility on dairy cows' intake, milk production, and body weight change. The dataset compiled for the study comprised 29 experiments with 96 diets, published in the literature since 1999. Average forage dietary concentration was 53.9% dry matter (DM) basis (ranged from 40.0 to 98.0%). Corn silage represented 77.6% (58.6–100%) of the total forage, for a total concentration in the diet DM of 42.0% (26.8–98.0%).

Daily milk yield and body weight gains increased respectively across studies 84 and 12 g/day for every one-percentage point increase in corn silage fiber (aNDFom) digestibility. Surprisingly, fiber digestibility did not significantly alter DM intake. Since corn silage was not the only ingredient in the diets, these effects would have been 1.29 greater if forage had consisted of only corn silage, and 2.38 greater if whole rations had been only corn silage. In conclusion, digestible fiber is an important parameter to consider when feeding corn silage to dairy cows.

Mounting feed costs and consumer concerns about the use of growth promoters and antibiotics in livestock production, provide ample incentive to revisit and refine the use of enzyme additives in ruminant diets. These products can improve feed conversion efficiency and reduce the cost of milk production²⁶. Feed additives with enzymatic fibrolytic activity offer a potential to enhance forage digestion, feed efficiency¹⁷, and income over feed costs (IOFC). Applying a blend of cellulase and xylanase enzyme products to forages (corn silage and alfalfa hay) prior to feeding 55:45 forage to concentrate diets, increased daily IOFC per cow from \$0.32 to \$0.88⁴⁰. When combining data from 20 studies and 41 treatments that added fibrolytic exogenous enzymes to dairy cow diets, Beauchemin et al.⁸ reported overall increases of 1.0 ± 1.3 kg/d and 1.1 ± 1.5 kg/d in DMI and milk yield, respectively. From the standard deviations is clear that responses to adding fibrolytic enzymes to dairy cow diets have been variable²⁶. This variability is not surprising, given that most of the commercially available enzyme products evaluated as ruminant feed additives are developed for non-feed applications¹³.

A meta-analysis on the effect of dietary application of exogenous fibrolytic enzymes on the performance of dairy cows was published recently in the *Journal of Dairy Science*. University of Florida's researchers⁴ included in the meta-analysis 15 peer-reviewed studies with 17 experiments and 36 comparisons. The most commonly used exogenous fibrolytic enzymes was a cellulase-xylanase complex (13 studies). Across all studies, feeding exogenous fibrolytic enzymes did not affect dry matter intake nor feed efficiency but tended to increase dry matter and fiber digestibility by relatively small amounts (1.36 and 2.30%, respectively). Enzyme application increased slightly milk yield (0.9 kg/day), 3.5% fat-corrected milk (FCM; 0.5 kg/day), and milk protein (0.03 kg/day). Surprisingly, increasing the rate of application of exogenous fibrolytic enzymes did not affect performance.

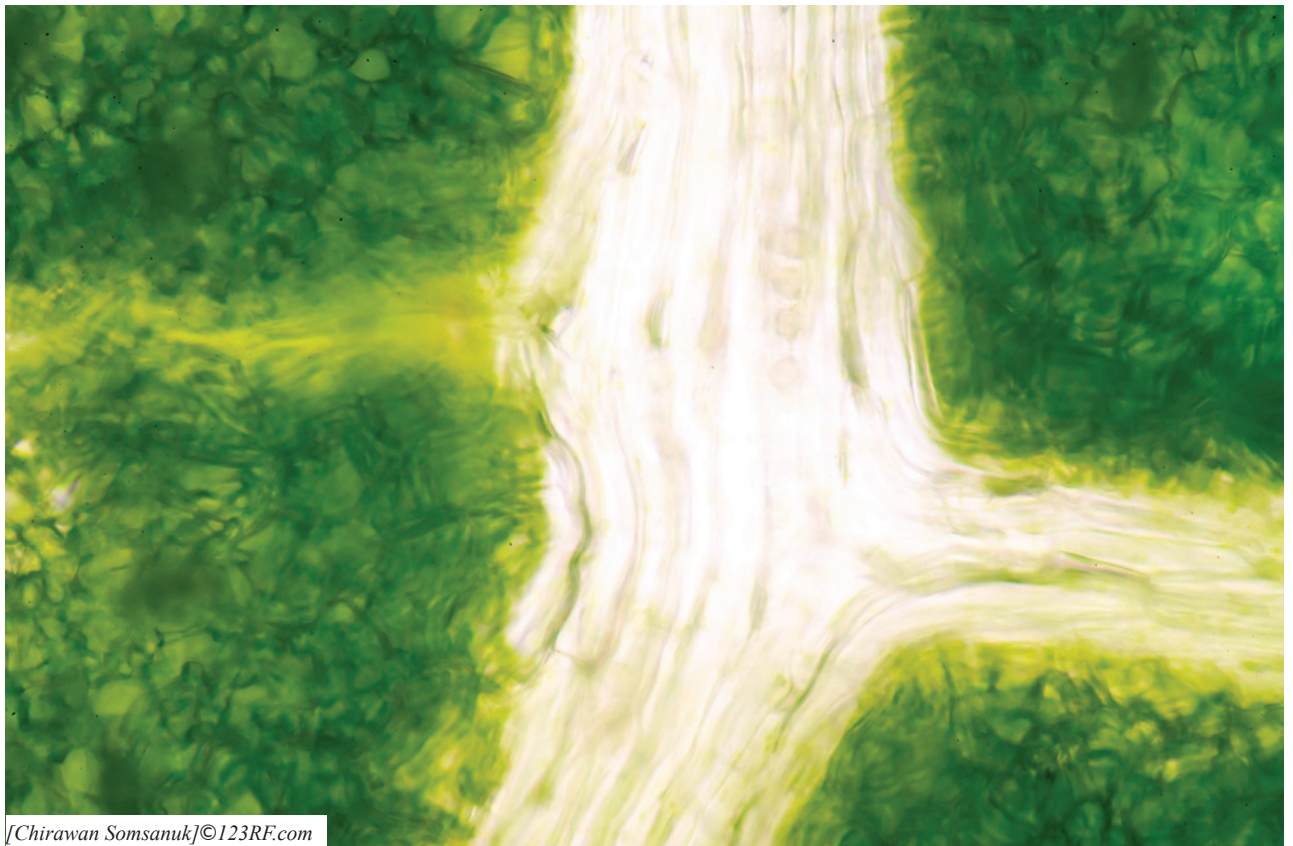
The use of feed enzyme additives in ruminant diets had slowed-down until recently given their relatively high cost, inconsistent response, and potential for improving animal performance with other emerging technologies. Higher costs of livestock production however, combined with the availability of newer enzyme preparations prompted a renewed interest in the potential of feed enzymes for ruminants⁴⁶. The total feed enzyme market quadrupled during the first decade of the 21st century. The split in their use by species has remained relatively similar, with sales highest for poultry, followed by swine, with the ruminant market still in its infancy¹¹. Feed enzymes for ruminants contain mainly cellulase and hemicellulase activities and are of fungal (mostly *Trichoderma longibrachiatum*, *Aspergillus niger*, *A. oryzae*) and bacterial (*Bacillus* spp.) origin³⁵.

Improvements in animal performance due to the use of feed enzymes have been

attributed to increases in feed digestion^{8,36}. Fibrolytic enzyme application enhanced DM (4 - 12%) and fiber (7 - 40%) digestibility in lactating dairy cows^{3,24,38}. Three main factors complicate explaining the mechanisms by which fibrolytic enzymes increase digestion and utilization of feedstuffs in ruminants¹⁸. First, feeds are structurally very complex, containing a variety of polysaccharides, protein, lipids, lignin, and phenolic acids, often in close association. Second, enzyme additives are usually blends of enzymes with many different actions, each of which differ in optimal conditions and specificities. Finally, ruminal fluid is by nature an extremely complex microbial ecosystem, containing multiple microbial species and their enzymes. Attempting to identify the individual mode of action of enzymes under such conditions would be nearly impossible.

The objective in this article is to review research trials that evaluated the effectiveness of fibrolytic enzymes feed additives on dairy cows' intake, milk yield and milk composition, feed efficiency, and body reserves. Performance data were obtained from 28 scientific articles published between 1999 and 2016, which studied the effects of dietary addition of fibrolytic enzyme products on the performance of lactating dairy cows (References: 2, 3, 9, 10, 11, 12, 14, 15, 17, 20, 21, 22, 24, 26, 29, 30, 31, 32, 33, 34, 37, 38, 39, 40, 41, 43, 46, 47, 49). It included 32 trials and 109 treatments conducted on research stations and commercial dairy farms.

All studies evaluated exogenous fibrolytic enzyme products with cellulase and xylanase activities, except those of Bernard et al.¹² and Knowlton et al.²⁹ which supplemented exclusively cellulases, and Mohamed et al.³⁴ which contained exclusively xylanases. In addition to cellulase and xylanase activities, some enzyme complexes contained ferulic acid esterase^{3,21}, amylase^{20,24}, pectinase¹⁰, or protease activities^{20,24}.



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Enzyme application

Enzymes were added to the diet at feeding time or only a few hours before; therefore, this review does not include trials in which enzymes were applied to forage at ensiling. Several methods of adding enzymes to the diets were used across the studies (Table 1). Enzyme products were applied to different portions of the diets including forage, concentrate, or complete TMR either in liquid (81.2%) or in powder forms (18.8%). Furthermore, some experiments compared these different methods of feeding enzymes to dairy cows^{2, 15, 41, 43, 46, 47}. Adesogan et al.² compared the effects of applying a fibrolytic enzyme product to the concentrate, forage, or TMR, whereas Bowman et al.¹⁵ blended it in different fractions of the TMR (concentrate, supplement or premix). Other studies tested the enzyme product either sprayed over the TMR or the concentrate^{41, 47}. In addition, Yang et al.⁴⁶ compared diets including forage treated or both forage and concentrate treated with a fibrolytic enzyme mixture.



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Effects on feed intake

Fibrolytic enzyme applied to dairy cow diets is often accompanied by increased feed intake. This can be attributed to increased palatability due to sugars released by pre-ingested fiber hydrolysis and/or post-ingested enzyme effects that result in improved fiber digestibility, gut fill reduction, and increased feed intake¹.

Fiber-digesting enzymes increased DMI by 0.9 - 3.2 kg/day in 5 out of 32 of the trials examined (15.6%; Table 2). On the other hand, feeding a non-commercial novel enzyme product with endoglucanase and xylanase activities at a high concentration decreased DMI of cows in early lactation²⁶ (Table 2). No other experiments have reported decreased DMI which makes the results of this study difficult to explain.

Table 1. Method of enzyme inclusion in the diets

		PORTION TO THE DIET TO WHICH ENZYMES ARE APPLIED		
		Forage	TMR	Concentrate
Presentation Form	Powder		Dehghani et al.2011 Elwakeel et al.2007 Gado et al.2009 Mohamed et al.2013	Knowlton et al.2002 Bilik et al.2009
	Liquid	Dhiman et al.2002 Kung et al.2000; year 1 Kung et al.2000; year 2 Kung et al.2002 Lewis et al.1999; trial 1 Lewis et al.1999; trial 2 Schingoethe et al.1999 Zheng et al.2000	Arriola et al.2011 Bernard et al.2010 Beauchemin et al.1999 Chung et al.2012 Holtshausen et al.2011 Peters et al.2015; trial 1 Peters et al.2015; trial 2 Romero et al.2016	Beauchemin et al.2000 Bowman et al.2002 Miller et al.2008 Rode et al.1999
		Adesogan et al.2007 Sutton et al.2003	Vicini et al.2003; trial 1 Vicini et al.2003; trial 2	Yang et al.1999 Yang et al.2000

Dry matter intake of cows was not affected when 2.5 g/kg of DM of an enzyme blend of cellulases and xylanases was added to the TMR; however, when the inclusion rate was doubled DMI increased by 14.3% (Dehghani et al.; 2011). In contrast, in other experiments (Beauchemin et al., 2000; Lewis et al., 1999, Trial 2) DMI increased regardless of the enzyme concentration. Moreover, the effect of the addition of a fibrolytic enzyme formulation to diets of dairy cows varied with stage of lactation. Lewis et al. (1999, Trial 2) detected interactions of week × treatment with increased DMI with enzyme addition, in weeks 3 to 7 but not weeks 8 to 16 of lactation. This difference observed on feed intake between early and late lactation may be due to the effects of ruminal fiber digestibility on feed intake²⁹.

In early lactation, cows are usually in negative energy balance, suggesting fill, and not energy demand, regulates intake. Late lactation cows on the other hand are usually in positive energy balance, suggesting energy demand, and not fill, regulates DMI. Therefore, intake of later lactation cows is less affected by increasing ruminal fiber digestibility because rumen fill does not limit intake²².

Table 2. Effects of fibrolytic enzymes on dry matter intake

Author/s (year)	Treatments	DMI (kg/d)
Beauchemin et al. (2000)	Control LE (1.22 L/t) HE (3.67 L/t)	20.5 ^a 22.0 ^b 21.6 ^b
Dehghani et al. (2011)	Control Enz1 (2.5 g/kg) Enz2 (5.0 g/kg)	22.3 ^a 23.9 ^a 25.5 ^b
Gado et al. (2009)	Control E (40 g/d)	16.1 ^a 18.2 ^b
Holtshausen et al. (2011)	Control LE (0.5 mL/kg) HE (1.0 mL/kg)	24.5 ^a 22.9 ^a 22.2 ^b
Lewis et al. (1999; trial 2)	Control LE (1.25 mL/kg) ME (2.50 mL/kg) HE (5.00 mL/kg)	24.4 ^b 26.2 ^a 26.2 ^a 26.6 ^a
Romero et al. (2016)	Control 2A (1.0 mL/kg) 3A (3.4 mL/kg)	22.6 ^a 23.5 ^b 22.5 ^a

^{a,b}Means with different letters within each study are different (P < 0.05).



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Effects on milk yield

Lactation responses of dairy cows fed fibrolytic enzymes have been scarce and inconsistent. The effectiveness of fibrolytic enzymes to improve milk production was observed only on 28% of the studies (Table 3). Across these 8 experiments that showed positive results, the increment in milk yield due to enzyme addition ranged from 1.2 to 6.3 kg/day. None of the experiments included in this literature review reported reduction on milk yield when cows were fed fibrolytic enzymes. Moreover, the response was highly dependent on enzyme dosage, enzyme combination, and method of enzyme application to the diets.

Kung et al.³¹ studied the effects of a carboxymethyl cellulase (CMCase) and xylanase complex at two different concentrations on milk production in dairy cows. Surprisingly, the authors reported that enzyme treatment at low (3500 CMCase and 16,000 xylanase units per kg of forage DM), but not high (8800 CMCase units and 40,000 xylanase units) concentrations improved milk production by 6.8%. Similarly, supplementing a fibrolytic enzyme mixture enhanced milk production by 3.2% at a low dosage rate (2.5 g/kg of DM) but not at a higher concentration (5 g/kg of DM; Dehghani et al., 2011). These results contrast with those of another experiment in which milk yield was greater in cows fed a diet that contained alfalfa hay cubes treated with 2 g per kg of hay of an enzyme blend; a lower enzyme application rate (1 g/kg hay) however was ineffective⁴⁶. Milk production was enhanced by 6.3 kg/d in early lactation cows receiving

an enzyme solution containing cellulases and xylanases at a rate of 2.5 ml/kg of forage DM (Lewis et al. 1999; Trial 2); however, milk yield did not increase in cows fed lower and higher amounts of enzymes (1.25 and 5.0 ml/kg of forage DM, respectively). The lack of response at low concentrations indicates insufficient dietary enzyme activity; however, the rationale for reduced enzyme response when added at higher rates of supplementation is less evident⁷. Adesogan¹ suggested three possible hypothesis for the lack of response when enzymes were used at high doses: first, it may be partially attributed to negative feedback inhibition of the enzyme-substrate interaction that occurs when enzyme action is inhibited by the increased concentration of a product. Second, fermentation of sugars produced by cell wall hydrolysis may reduce ruminal pH to levels that inhibit cell wall digestion. Third, it is possible that exogenous enzymes compete with the rumen population for cellulose binding sites available on feeds. This latter process could explain the lack of response usually reported with enzymes used at higher doses⁷. The fact that it is possible to either overfeed or underfeed enzymes makes their application complex¹⁹, and emphasizes the need to determine optimal concentrations of enzyme additions necessary for any given feeding situation³².

The source and combination of specific enzymes is also an important factor in improving lactation response. Kung et al.³¹ compared the effect on milk production of two different cellulase-enzyme complexes derived from different fermentations of the same organism combined with a single xylanase-enzyme complex (Table 3). Milk production was similar for cows fed untreated forage or forage treated with the enzyme complex EA2 (3700 carboxymethyl cellulase and 14,000 xylanase units) however, milk production increased by 2.5 kg in cows fed EB1.2 (3600 carboxymethyl cellulase and 11,000 xylanase units).

Table 3. Effects of fibrolytic enzymes on milk yield

Author/s (year)	Treatments	DMI (kg/d)
Dehghani et al. (2011)	Control Enz1 (2.5 g/kg) Enz2 (5.0 g/kg)	37.9 ^a 39.1 ^b 36.7 ^a
Gado et al. (2009)	Control E	12.8 ^a 15.7 ^b
Kung et al. (2000; year 1)	Control EA2 (2 L/T) EA5 (5 L/T)	37.0 ^A 39.5 ^B 36.2 ^A
Kung et al. (2000; year 2)	Control EA2 EB1.2	32.9 ^A 33.6 ^{A,B} 35.4 ^B
Lewis et al. (1999; trial 1)	Control E	25.9 ^a 27.2 ^b
Lewis et al. (1999; trial 2)	Control LE (1.25 ml/kg) ME (2.50 ml/kg) HE (5.00 ml/kg)	39.6 ^b 40.8 ^b 45.9 ^a 41.2 ^b
Mohamed et al. (2013)	Control E	39.5 ^a 41.0 ^b
Yang et al. (1999)	Control LH (1 g/kg) HH (2 g/kg) HT (2 g/kg)	23.7 ^b 24.6 ^{a,b} 25.6 ^a 25.3 ^{a,b}
Yang et al. (2000)	Control E-TMR E-Conc	35.3 ^b 35.2 ^b 37.4 ^a

^{a,b} Means with different letters within each study are different (P < 0.05).

^{A,B} Means with different letters within each study are different (P < 0.10).

Two studies conducted by a Canadian research group showed that the lactation response of dairy cows fed fibrolytic enzymes depended on the portion of the diet to which the enzyme complexes was applied. Yang et al.⁴⁶ reported an 8% increase in milk production in cows fed alfalfa hay cubes treated with 2 g per kg of an enzyme supplement compared with untreated cubes. There was no response however, in milk yield when both concentrate and cubes were treated with 1 g per kg of DM of the same enzyme complex. In a subsequent experiment⁴⁷, milk yield was 2.1 kg/d higher in cows

fed a commercial enzyme product added to the concentrate than cows fed a control diet, whereas applying the same enzyme complex to the TMR did not affect milk production. It has been suggested that enzymes applied to a TMR immediately prior to feeding, may be released into the rumen fluid and pass rapidly to the lower tract before they can be effective in the rumen¹⁰.

Increased post ruminal digestion due to enzyme supplementation of the TMR may improve apparent digestibility in the total tract without increasing milk production. This can be attributed to the ruminal fermentation providing the host animal with energy in the form of VFA, and amino acids in the form of bacterial protein, whereas hindgut fermentation can only supply energy⁴⁷.



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Effects on milk fat yield

Milk fat of cows fed enzymes increased in only 3 out of 22 experiments included in this literature review (Table 4). In one experiment³² (trial 2) fat yield increased as a result of higher milk production. In another experiment however⁴⁰ it was due to an increase in milk fat concentration. In a third study⁴⁹ however; higher milk fat yields in cows fed enzyme-treated diets were not accompanied by significant increases in either milk yield or milk fat concentration.

When enzyme-treated forages were fed milk fat production did not depend on either enzyme dosage or stage of lactation. Schingoethe et al.⁴⁰ reported greater milk fat

yield when feeding increased amounts of a cellulase-xylanase enzyme blend, but these differences were not significant ($P > 0.2$; Table 4). Zheng et al.⁴⁹ found no advantage for start feeding enzyme-treated forages in the close-up dry period or at calving compared with starting at peak milk production (Table 4).

Table 4. Effects of fibrolytic enzymes on milk fat yield

Author/s (year)	Treatments	DMI (kg/d)
Lewis et al. (1999; Trial 1)	Control Enzyme	0.91 ^a 0.99 ^b
Schingoethe et al. (1999)	Control LE (0.7L/t) ME (1.0 L/t) HE (1.5 L/t)	0.90 ^a 0.98 ^b 1.03 ^b 1.08 ^b
Zheng et al. (2000)	Control E12wk E18wk E24wk	1.36 ^a 1.47 ^b 1.55 ^b 1.44 ^b

^{a,b} Means with different letters within each study are different ($P < 0.05$).

Milk fat is synthesized from fatty acids from the peripheral circulation (60%) or synthesized de novo in the mammary gland¹⁶ (40%). In ruminants, short- and medium-chain fatty acids (4 to 14 carbons), and a portion of the 16-carbon fatty acids derive from de novo synthesis from acetate and to a lesser extent β -hydroxybutyrate²⁵. Acetate is produced in the rumen from carbohydrate fermentation, whereas β -hydroxybutyrate is produced in the rumen epithelium from absorbed butyrate⁵. Preformed fatty acids, originate from absorption from the digestive tract or mobilization from body reserves, and account for the remaining 16-carbon and all of the longer-chain fatty acids (>16 C), and are taken up from the circulating plasma pool².

There seems to be inconsistency in the literature on the effects of fibrolytic enzymes on ruminal fermentation. Although enzyme application to dairy cow diets increased total VFA concentration^{3, 24} and the proportion of acetate in ruminal fluid²⁴, most studies have reported no effect of enzymes on concentration of total VFA, and molar proportions of acetate and butyrate in ruminal fluid^{10, 15, 17, 27, 36, 46}. Moreover, Sutton et al.⁴¹ reported a decrease in the molar proportion of acetate in the ruminal fluid in cows fed a fibrolytic enzyme product with xylanase and endoglucanase activities. Therefore, it can be suggested that the lack of effectiveness for enzymes to increase the proportions of acetate and butyrate may explain the parallel response in milk fat yield reported in most studies included in this literature review.

Effects on milk protein yield

Significant increases in milk protein yield due to fibrolytic enzyme addition to dairy diets were observed in five out of the 23 experiments that reported milk protein yield (Table 5). In other studies^{15,40} milk protein content improved with the inclusion on fibrolytic enzymes; however, milk protein yield was unaffected.

Table 5. Effects of fibrolytic enzymes on milk protein yield.

Author/s (year)	Treatments	Protein (kg/d)
Gado et al. (2009)	Control E	0.45 ^a 0.57 ^b
Lewis et al. (1999; trial 1)	Control E	0.82 ^a 0.88 ^b
Mohamed et al. (2013)	Control E	1.30 ^a 1.36 ^b
Sutton et al. (2003)	Control TE CE	1.13 ^b 1.18 ^a 1.18 ^a
Zheng et al. (2000)	Control E12wk E18wk E24wk	1.00 ^a 1.08 ^b 1.15 ^b 1.08 ^b

^{a,b} Means with different letters within each study are different ($P < 0.05$).

Lewis et al.³² (trial 1) and Mohamed et al.³⁴ reported greater milk protein yield as a result of increased milk production in cows receiving the enzyme product. These data are consistent with those of Gado et al.²⁴ who observed 27% improvement in milk protein output, as a result of higher milk yields (+2.9kg/d.) and higher protein concentration (+0.01 percentage units) when diets were supplemented with enzymes. Zheng et al.⁴⁹ also recorded higher milk protein production in cows fed enzyme-treated forages. In that experiment however, milk protein percentage and milk production were unaffected. Furthermore, the authors did not find differences on the time of introducing the enzyme, whether immediately after calving, or at peak production.

Sutton et al.⁴¹ evaluated the effect of method of application of a fibrolytic enzyme product on performance of early lactation cows. The enzyme enhanced milk protein yield similarly (50 g/d) either if it was sprayed on the TMR or on the concentrate. The use of enzymes has been associated with improved efficiency of microbial protein

synthesis in the rumen²⁴. Using a fibrolytic enzyme product, these authors increased microbial nitrogen synthesis in enzyme-supplemented cows (220 vs. 190 g/d). In that study increased milk yield by enzyme addition may be partly due to increased microbial nitrogen synthesis in the rumen. This theory however is not supported by other studies with enzyme-supplemented lactating cows, which reported no improvement in both flow of microbial nitrogen passing to the duodenum^{10, 27, 46} or synthesis of microbial nitrogen within the rumen¹⁵.

Effects on feed efficiency

Feed costs represent the highest expense to dairy producers and, consequently, maximizing utilization of nutrients is essential to the profitability and sustainability of dairy farms. With feed comprising the largest operating expense in the production of milk, efficiency of converting feed to milk is a key element to assess profitability of dairy operations. Feed efficiency, is usually defined as the ratio of milk output to feed input, namely, milk yield to feed intake. Since, feed efficiency is a ratio of two metrics, cows with higher milk production, lower intake, or both may be more efficient.

Feeding of fibrolytic enzymes has been proposed as one way to increase efficiency due to higher nutrient bioavailability in feeds^{17,32}. However, data obtained from studies on dairy cows are not promising. In these studies, milk output was defined as yield in kg, FCM or energy corrected milk (ECM), while feed input was expressed as kg of DMI. Only 3 out of 21 experiments reported increments in feed efficiency when fibrolytic enzymes were fed to dairy cows (Table 6). Improvements in feed efficiency in two of the experiments^{3, 26} was due to lower DMI without changes in milk production. In the third study³⁴ however cows fed enzymes were more efficient because they produced more milk.

Diet composition and enzyme dose influenced feed conversion efficiency in these studies. Arriola et al.³ reported significant increase in milk production efficiency (FCM/DMI; 1.46 vs. 1.62) in cows fed a low concentrate diet (33% concentrate DM basis) treated with a fibrolytic enzyme. The authors however, did not find an effect in those fed high concentrate (48%). Milk production efficiency (kg of milk/kg of DMI) and FCM production efficiency (kg of FCM/kg of DMI) increased linearly with increasing enzyme addition²⁶. Cows in early lactation fed an enzyme added to the diet at a high concentration (1.0 mL/kg of TMR DM) increased FCM production efficiency by 11.3%. At a lower enzyme concentration (0.5 mL/kg of TMR DM) however, FCM production efficiency did not improve, with the responses being numerically intermediate (5.3%) compared to those in the control and enzyme-treated diets at the higher dose.

Table 6. Effects of fibrolytic enzymes on feed efficiency

Author/s (year)	Treatments	Kg milk/DMI
Arriola et al. (2011)	Control	1.46 ^a
	Low-conc.E	1.69 ^b
	Control	1.42
	High-conc.E	1.51
Dehghani et al. (2011)	Control	1.60 ^a
	Enz1 (2.5 g/kg)	1.51 ^a
	Enz2 (5.0 g/kg)	1.33 ^b
Holtshausen et al. (2011)	Control	1.50 ^a
	LE (0.5 ml/kg)	1.58 ^a
	HE (1.0 ml/kg)	1.67 ^b
Lewis et al. (1999; trial 1)	Control	1.28 ^a
	E	1.21 ^b
Lewis et al. (1999; trial 2)	Control	1.82 ^a
	LE (1.25 ml/kg)	1.64 ^b
	ME (2.50 ml/kg)	1.86 ^a
	HE (5.00 ml/kg)	1.62 ^b
Mohamed et al. (2013)	Control	1.58 ^a
	E	1.64 ^b

^{a,b} Means with different letters within each study are different (P < 0.05).

On the other hand, three studies (17.6%) reported reductions in feed efficiency when fibrolytic enzymes were supplemented^{20,32} (Table 6). Lewis et al.³² observed decreased feed efficiency in both early- and mid- lactation cows receiving forage sprayed with a solution containing cellulases and xylanases. Production efficiency of ECM in early lactation cows consuming forage treated with a medium-enzyme dose (1.86; Table 6) was similar to that of cows fed untreated forage (1.82). Milk production efficiency however was lower in cows fed forage treated at low- (LE; 1.64) and high-enzyme dosages³² (HE; 1.62; Trial 2). Milk production did not differ despite the fact that both LE and HE groups consumed more DMI than control cows. The authors suggested that greater DMI might have been partitioned to body reserve accretion rather than milk production. Dehghani et al.²⁰ also reported lower feed efficiencies when cows were fed a commercial fibrolytic enzyme at high rates (5.0 g/kg) with no effect observed at a lower dose (2.5 g/kg). These results indicate the importance of determining optimal enzyme concentrations to be included into diets.

Effects on body reserves

Dairy cows mobilize body tissues to support energy requirements for milk production during early lactation and replenish tissue reserves for the subsequent lactation during mid and late lactation. Fibrolytic enzyme supplementation had little impact on body reserves of lactating dairy cows. Only one study out of twenty-one²⁹ reported higher BW gains when a commercial enzyme formulation was fed to lactating dairy cows. In this study, cows fed diets containing enzymes gained more weight than those on the untreated diet (+0.60 vs. -0.03 kg/day). Moreover, an interaction between stage of lactation and enzyme treatment was observed due to the change in weight gain being greater in early lactation (+1.16 kg/day) than in late lactation cows (+0.10 kg/day).

In contrast, Elwakeel et al.²² reported lower BW gains when cows were fed fibrolytic enzymes at high dose compared with control cows. Moreover, BW changes demonstrated a quadratic effect because weight gain was less for cows receiving intermediate amounts of a fibrolytic enzyme (5 and 10 g/d) than those fed either no enzyme or at the highest amount (15 g/d). It is surprising that in some experiments increased total tract DM and NDF digestibility of the diets due to enzyme supplementation did not affect BW change, given that milk or milk components (fat and protein) yield were also not affected^{2, 3, 38}.



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Effects of experimental design

No effects of fibrolytic enzymes were found on intake, milk production, feed efficiency, or body reserves of dairy cows in 12 out of 32 experiments (References: 2, 10, 12, 14, 17, 21, 30, 32, 37, 38, 43). Lack of statistical differences in cow performance among diets may be attributed to inappropriate experimental design. In 10 of the studies examined in this review the experimental design was a Latin Square. Animal responses that require longer periods of time to manifest will not be detected with this design, and carryover effects that last more than the 14 days adaptation period could confound the results²². Schingoethe et al.⁴⁰ indicated that dairy producers may not observe the maximal response to fibrolytic enzyme products within the 1st week of use, but responses should be apparent in 2 to 4 weeks. Two parallel studies conducted at the University of Alberta using the same basal diets and fibrolytic enzyme treatments obtained different results^{17,26}. Using a complete randomized block design with 9- to 10-week periods Holtshausen et al.²⁶ reported increment in milk production efficiency of lactating dairy cows fed a commercial fibrolytic enzyme blend because of a lower level of DMI with no change in milk production.

Chung et al.¹⁷ however did not find effects on DMI stating the Latin Square design and relatively short periods (3 weeks per period) used in their study may have been inadequate to evaluate the effects of the enzyme on milk production efficiency. Some numerical ($P > 0.05$) responses may have masked real effects because lack of significance in additive evaluation trials sometimes reflect inadequate replication of experimental units and a consequent lack of power to detect treatment differences².

Conclusions

Attempts to improve dairy cow performance with fibrolytic enzymes applied to the feed at or only hours before feeding have yielded variable production responses. This inconsistency may be due to variable forage-to-concentrate ratios, application rates, lactation stage, fraction of the diet to which enzymes were applied, and enzyme combinations. At the present time the use of fibrolytic enzymes in commercial dairy farms seems not cost effective given the relatively high price of enzyme additives, their inconsistent response, and the potential of improving cow performance with other additives. More research is required using long term studies to provide more consistent and reliable results of the economic impact of feeding fibrolytic enzymes to dairy cows.

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