Influence of fibrolytic enzymes on ruminal disappearance and fermentation in steers fed diets with short and long particle length of forage

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Abstract

The effects of an exogenous fibrolytic enzyme mixture on in sacco ruminal disappearance and fermentation of growing (380 kg body weight) and finishing (440 kg body weight) steers fed with diets to short (10 mm) and long (50 mm) particle length (PL) were evaluated. Four Holstein steers fitted with ruminal cannulas were randomly assigned to: (1) diet with short PL forage; (2) diet with short PL forage + enzyme; (3) diet with long PL forage; (4) diet with long PL forage + enzyme.

Fibrolytic enzymes did not affect dry matter intake. In growing steers, potentially disappearance fraction and total disappearance of DM, starch, NDF and ADF were increased by enzymes. In finishing steers, enzymes increased potentially disappearance fractions and total disappearance of DM, starch, NDF and ADF. In both growing and finishing steers, enzymes increased ruminal ammonia concentrations.

Enzymes increased ruminal disappearance of diets for steers, but it had scarce effects on ruminal fermentation and intake.

Introduction

The beef cattle industry has changed dramatically in the last decades. Currently, beef steers are fed with high-grain diets to provide an energetically high-density ration. However, these diets need dietary forage having an adequate particle size to enhance ruminal digesta stratification, chewing activity and ruminal buffering (Zebeli et al., 2008). The site and extent of digestion of feedstuffs dietary components in beef cattle is a function related to digestion rate and to the staying time in each segment of the digestive tract (Ewing and Johnson, 1987). Grinding forages generally decreases extent of ruminal digestion, but enhance the digesta stratification and chewing activity, the available surface area for attachment of ruminal bacteria, and feed utilization efficiency in domestic ruminants (Van Soest, 1994; Yang and Beauchemin, 2006). Recent research confirms that ruminal activities of nonstarch polysaccharide-degrading enzymes were increased as particle length (PL) of corn silage reduced, and thus a greater fibrolytic activity of rumen digesta with decreasing PL were found (Zebeli et al., 2008). Using this approach, we hypothesized that exogenous fibrolytic enzymes action may also be affected by PL of forage and therefore increase dry matter intake and digestion. Therefore, the objectives of this study were to evaluate the effects of exogenous fibrolytic enzymes on in sacco disappearance and fermentation of steers fed on diets with short or long PL of forage.

Materials and methods

The study was conducted at the Colegio de Postgraduados, Campus Montecillo in México. Procedures were approved and supervised by an Academic Committee of The Department of Animal Science of Colegio de Postgraduados according to regulations established by the Animal Protection Law enacted by the State of México.

Four Holstein steers fitted with ruminal cannulas were randomly assigned to one of four diets (treatments) being: (1) diet with short PL of forage; (2) diet with short PL of forage + enzyme; (3) diet with long PL of forage; (4) diet with long PL of forage + enzyme. Experimental diets were the conventional one used by smallholder cattle growers of the State of México. Corn stover and alfalfa hay were asseded through a hammer mill (Azteca, Guadalajara, Jalisco in México) fitted with a 10 mm screen for short PL or with a 50 mm screen for long PL.

One kilogram of ground forage (a mixture of 500 g of corn stover + 500 g of alfalfa hay) was sprayed with 3 g of enzyme powder preparation. These three grams of enzyme powder preparation were dissolved in 300 mL of distilled water and applied in a fine spray to the alfalfa-corn stover mixture 24 h before diurnal feeding time. The same amount of water was sprayed daily on the control diet. The forage mixtures were added to the total mixed rations.

According to Giraldo et al. (2008), at pH 6.5 and 39°C the fibrolytic enzyme powder preparation from Aspergillus niger and Trichoderma viride (Fibrozyme, Altech Inc., Nicholasville, KY, USA) liberated 148 µmol of glucose from carboxymethylcellulose and 791 µmol of xylose from oat spelt xylan per minute and no exogluco-canse and amylase were detected.

Diets and water were offered at 7:30 and 19:30 h. Steers had free choice access to diets (Table 1). The study had two experimental phases, as follows: phase 1, defined as “growing trial”, in which steers were weighing 380 kg ± 20 kg body weight at the beginning of the phase; phase 2, defined as “finishing phase”, in which steers were weighing 440 ± 19 kg at the beginning of the phase. Each phase consisted of four 20 d periods, each counting 15 d for adaptation and 5 d for sampling ruminal fluids, in sacco disappearances, intake and BW change.

The DM was determined by oven drying at 78°C.

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Table 1. Ingredients and chemical composition of experimental diets*.

| Ingredients, g/kg DM | Growing phase | | | | | Finishing phase | | | |
|---------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
|                     | Short-ENZ     | Short+ENZ     | Long-ENZ      | Long+ENZ      | Short-ENZ     | Short+ENZ     | Long-ENZ      | Long+ENZ      |
| Alfalfa hay         | 250           | 250           | 250           | 250           | 174           | 174           | 174           | 174           |
| Corn stover         | 250           | 250           | 250           | 250           | 174           | 174           | 174           | 174           |
| Sorghum grain       | 366           | 366           | 366           | 366           | 505           | 505           | 505           | 505           |
| Soybean meal 440 g, CP/kg, DM | 88 | 88 | 88 | 88 | 61 | 61 | 61 | 61 |
| Poultry litter      | 24            | 24.25         | 24            | 24.25         | 64            | 63.25         | 64            | 63.25         |
| Mineral premix      | 22            | 22            | 22            | 22            | 22            | 22            | 22            | 22            |
| Enzymes             | 0.75          | 0.75          | 0.75          | 0.75          | 0.75          | 0.75          | 0.75          | 0.75          |
| Chemical composition |               |               |               |               |               |               |               |               |
| Dry mater, g/kg     | 899           | 889           | 882           | 883           | 888           | 901           | 900           | 894           |
| Crude protein, g/kg DM | 125         | 130           | 128           | 133           | 120           | 110           | 123           | 117           |
| Starch, g/kg DM     | 271           | 265           | 278           | 274           | 347           | 356           | 348           | 351           |
| Acid detergent fibre, g/kg DM | 322 | 302 | 329 | 307 | 273 | 270 | 276 | 261 |
| Acid detergent fibre, g/kg DM | 199 | 182 | 188 | 175 | 181 | 169 | 183 | 170 |

*Short particle length, 0.39 inches; long particle length, 1.97 inches; -ENZ, without enzyme; +ENZ, with enzyme. *Content per kg: NaCl 992 g; Ca 100 g; P 40 g; Mg 8.7 g; I 110 mg; Zn 1.1 g; Fe 0.22 g; Cu 0.22 g; Co 0.01 g.

Results

In the growing phase, particle length (PL) of forage increased potentially disappearance fraction of DM and starch, without any effect on NDF and ADF fraction (Table 2). Soluble fractions and disappearance rates of DM, starch, NDF and ADF were affected by PL and enzyme. Potentially disappearance fraction and total disappearance of DM, starch, NDF and ADF were increased by enzymes (P<0.05). Interactions on total disappearance of DM and potentially disappearance fraction of starch indicate that enzymes had higher effect on ruminal disappearance of the diet with short than the one with long PL of forage.

Growing steers fed on diets with short or long PL had similar intakes. Dry matter intake was not affected by enzymes. Ruminal pH, volatile fatty acid (VFA) concentrations and fermentation efficiency were not affected by PL and enzyme. The only effect of enzymes was observed on ruminal ammonia concentration, which was increased (P<0.05) by this feed additive (Table 2).

In the finishing phase, particle length did not affect in sacco disappearance of DM and starch (Table 3). Potentially disappearance fraction
and total disappearance of NDF and ADF were higher in diets with short than long PL. Also, diets with long PL had lower soluble fraction and higher disappearance rate of ADF. Enzymes increased (P<0.05) potentially disappearance fractions and total disappearance of DM, starch, NDF and ADF. Enzymes did not affect soluble fractions and disappearance rates of DM, starch, NDF and ADF. There were no interactions observed between PL and enzymes on *in sacco* disappearances.

Daily intakes in the finishing steers were not affected by enzymes (Table 3). Steers fed on diets with short or long PL had similar ruminal fermentation characteristics and growth performance (Table 3). Enzymes induced higher (P<0.05) ammonia concentrations in steers fed on diets with long or short PL.

### Discussion

There were scarce effects of PL on intake and fermentation patterns. This finding agrees with Beauchemin and Yang (2005), and Zebeli *et al.* (2008), who reported similar intake and fermentation patterns with decreasing PL of corn silage in total mixed rations for dairy cows. Our results suggest some reduction on disappearance fractions of NDF and ADF as PL reduced. Indeed, decreasing PL of forage reduced fibre digestion (Yang *et al.*, 2002), although greater surface area per gram DM of ground forage should allow more rapid colonization by ruminal microbes and, subsequently, more extensive fermentation of short vs long PL (Firkins *et al.*, 1986).

Chemical composition of diets was relatively similar. The exception was for NDF and ADF contents, which were apparently reduced by enzymes. There are evidences that exogenous fibrolytic enzymes (Krause *et al.*, 1998; Pinos-Rodriguez *et al.*, 2008) applied to feed before feeding affect its composition, principally by increasing solubility of DM and NDF, possibly releasing more nutrients that are then available to enhance microbial colonization of feed particles (Yang *et al.*, 1999).

Our results showed that most of the positive effects of the fibrolytic enzymes were on the potentially disappearance fractions of DM, starch, NDF and ADF, without any influence on the potentially disappearance fraction of NDF. The fact that the enzymes have failed to increased the degradation rate of NDF in the current

### Table 2. Effect of fibrolytic enzymes and particle length on ruminal disappearance and fermentation of diets in growing steers°.

| Short | Long | Significance |
|-------|------|--------------|
| -ENZ  | +ENZ | -ENZ | +ENZ | SEM | PL | ENZ | INT |
| Dry matter intake, kg/d | 10.8 | 10.3 | 10.9 | 10.0 | 0.58 | ns | ns | ns |
| In sacco disappearance | | | | | | | | |
| Dry matter, g/kg | | | | | | | | |
| Soluble fraction | 237 | 223 | 187 | 168 | 4.2 | ns | ns | ns |
| Potential disappearance | 622 | 651 | 624 | 681 | 6.2 | * | ** | ns |
| Total disappearance | 859 | 874 | 811 | 849 | 9.4 | *** | * | * |
| Disappearance rate/h | 0.036 | 0.041 | 0.047 | 0.035 | 0.004 | ns | ns | ns |
| Starch, g/kg | | | | | | | | |
| Soluble fraction | 325 | 303 | 273 | 223 | 9.8 | ns | ns | ns |
| Potential disappearance | 626 | 662 | 679 | 743 | 14.4 | ** | * | * |
| Total disappearance | 951 | 965 | 952 | 966 | 3.0 | ns | * | ns |
| Disappearance rate/h | 0.059 | 0.057 | 0.062 | 0.060 | 0.35 | ns | ns | ns |
| Neutral detergent fibre, g/kg | | | | | | | | |
| Soluble fraction | 141 | 111 | 169 | 111 | 5.1 | ns | ns | ns |
| Potential disappearance | 447 | 534 | 425 | 531 | 9.9 | ns | *** | ns |
| Total disappearance | 588 | 645 | 594 | 642 | 8.1 | ns | * | ns |
| Disappearance rate/h | 0.031 | 0.032 | 0.028 | 0.022 | 0.005 | ns | ns | ns |
| Acid detergent fibre, g/kg | | | | | | | | |
| Soluble fraction | 109 | 117 | 138 | 136 | 6.3 | ns | ns | ns |
| Potential disappearance | 433 | 485 | 424 | 469 | 12.7 | ns | *** | ns |
| Total disappearance | 542 | 602 | 540 | 605 | 7.7 | ns | *** | ns |
| Disappearance rate/h | 0.022 | 0.021 | 0.026 | 0.022 | 0.005 | ns | ns | ns |
| Ruminal fermentation | | | | | | | | |
| pH | 6.13 | 6.5 | 6.36 | 6.49 | 0.10 | ns | ns | ns |
| N-NH3, mg/dL | 8.8 | 10.6 | 9.4 | 12.0 | 0.81 | ns | * | ns |
| Total VFA, nmol/L | 82.8 | 87.5 | 84.5 | 84.0 | 10.31 | ns | ns | ns |
| Acetate, mol/100 mol | 62.0 | 62.2 | 64.7 | 65.6 | 1.30 | ns | ns | ns |
| Propionate, mol/100 mol | 21.8 | 20.6 | 20.1 | 19.0 | 0.92 | ns | ns | ns |
| Butyrate, mol/100 mol | 16.2 | 17.2 | 15.2 | 15.4 | 0.81 | ns | ns | ns |
| Acetate:propionate ratio | 2.8 | 3.0 | 3.2 | 3.4 | 0.20 | ns | ns | ns |
| Fermentation efficiency, mol/100 mol | 73.4 | 75.6 | 73.3 | 73.9 | 0.40 | ns | ns | ns |

°Short particle length, 0.39 inches; long particle length, 1.97 inches; -ENZ, without enzyme; +ENZ, with enzyme; PL, particle length factor; ENZ, enzyme factor; INT, particle length x enzyme. ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.
study, as compared to our previous results (Pinos-Rodriguez et al., 2008), could be due to the different feed ingredients of the experimental diets, which could affect the enzyme mechanisms.

The increments of in sacco disappearance of starch by enzymes found in this study were an unexpected effect. Fibrolytic enzyme mixtures are not limited to the dietary component to which the enzymes are applied, which explains why fibrolytic enzymes can be effective in improving digestibility of the non-fibre carbohydrate fraction, in addition to increasing digestibility of the fibre components of diets (Beauchemin et al., 2003; Pinos-Rodriguez et al., 2008). Indeed, Pinos-Rodríguez et al. (2002) suggested that the same enzyme powder preparation, which is rich in xylanolytic activity (Giraldo et al., 2008), increased digestion of CP in addition to the apparent digestibility of NDF in alfalfa hay. This previous result found in our laboratory may explain in part why in the current experiments the enzymes did increase ruminal ammonia concentrations. Another good example that exogenous enzymes are not limited to a specific substrate was evidenced by Colombatto and Beauchemin (2009), who found that protease enzyme increased in vitro degradation of DM and ADF, and the disappearance of hemicelluloses of alfalfa. However, the reasons why fibrolytic enzymes increased starch disappearance are difficult to explain, even more that Giraldo et al. (2008) did not detect any exoglucoanase and amylase activities in the same fibrolytic enzyme powder preparation.

Our results showed few interactions between PL and enzyme, but a higher starch in sacco disappearance by enzymes was found with the shorter PL. Perhaps these exogenous enzymes had a greater activity of rumen digesta with decreasing PL of forage such as was found with native degrading enzymes (Zebeli et al., 2008). In addition, these results suggest that lowering the PL of forages may be beneficial due to increasing starch degradation in the rumen, particularly by increasing the activities of exogenous enzymes. Indeed, tendencies for increased rate of particle passage out of the rumen with enzyme-treated feeds have also been reported by Beauchemin et al. (1999) and Yang et al. (1999). It was argued by Beauchemin et al. (1999) that the enhanced rumen particle outflow rate was an explanation for the trends for a shift in the site of fibre digestion from the rumen to the hindgut in the presence of enzymes (Sutton et al., 2003). Further research is needed to elucidate effects of forage particle length and exogenous fibrolytic enzymes on passage and whole digestion.

Despite the potential benefits of using exogenous enzymes, the adoption of enzyme technology by the beef industry has been slow, due to the relatively high cost of enzyme products compared with ionophores, antibiotics, and implants (Beauchemin et al., 2003).

### Table 3. Effect of fibrolytic enzymes and particle length on ruminal disappearance and fermentation of diets in finishing steers°.

|                        | Short ENZ | +ENZ | Long ENZ | +ENZ | SEM | PL | ENZ | INT |
|------------------------|-----------|------|----------|------|-----|----|-----|-----|
| **Dry matter intake, kg/d** | 12.9      | 12.1 | 14.3      | 14.5 | 0.54| ns | ns  | ns  |
| **In sacco disappearance** |           |      |           |      |     |    |     |     |
| **Dry matter, g/kg**    |           |      |           |      |     |    |     |     |
| Soluble fraction        | 254       | 234  | 246       | 232  | 6.0 | ns | ns  | ns  |
| Potential disappearance | 588       | 640  | 576       | 615  | 14.8| ns **| ns **| ns  |
| Total disappearance     | 842       | 674  | 822       | 847  | 13.9| ns **| ns  | ns  |
| Disappearance rate /h   | 0.039     | 0.038| 0.036     | 0.036| 0.005| ns | ns  | ns  |
| **Starch, g/kg**        |           |      |           |      |     |    |     |     |
| Soluble fraction        | 330       | 259  | 284       | 210  | 18.7| ns | ns  | ns  |
| Potential disappearance | 651       | 731  | 688       | 780  | 12.2| ns **| ns  | ns  |
| Total disappearance     | 981       | 990  | 972       | 990  | 22.0| ns * | ns  | ns  |
| Disappearance rate /h   | 0.046     | 0.051| 0.047     | 0.063| 0.005| ns | ns  | ns  |
| **Neutral detergent fibre, g/kg** |     |      |           |      |     |    |     |     |
| Soluble fraction        | 119       | 157  | 129       | 168  | 12.2| ns | ns  | ns  |
| Potential disappearance | 418       | 446  | 352       | 413  | 13.1| * ***| ns **| ns  |
| Total disappearance     | 537       | 603  | 481       | 581  | 15.8| * ***| ns  | ns  |
| Disappearance rate /h   | 0.037     | 0.035| 0.029     | 0.030| 0.003| * | ns  | ns  |
| **Acid detergent fibre, g/kg** |   |      |           |      |     |    |     |     |
| Soluble fraction        | 71        | 94   | 104       | 116  | 7.2 | ns * | ns  | ns  |
| Potential disappearance | 463       | 488  | 370       | 445  | 16.1| * ***| ns **| ns  |
| Total disappearance     | 474       | 562  | 474       | 561  | 17.9| ns **| ns  | ns  |
| Disappearance rate /h   | 0.033     | 0.032| 0.026     | 0.029| 0.004| * | ns  | ns  |
| **Ruminal fermentation**|           |      |           |      |     |    |     |     |
| pH                      | 6.3       | 6.5  | 6.0       | 6.2  | 0.14| ns | ns  | ns  |
| N-NH3, mg/dL            | 12.4      | 16.6 | 13.2      | 17.5 | 1.14| ns * | ns  | ns  |
| Total VFA, mmol/L       | 96.4      | 103.0| 100.8     | 103.4| 7.17| ns | ns  | ns  |
| Acetate, mol/100 mol    | 61.8      | 67.5 | 62.0      | 64.4 | 2.90| ns  | ns  | ns  |
| Propionate, mol/100 mol | 21.5      | 17.4 | 20.9      | 20.2 | 1.71| ns  | ns  | ns  |
| Butyrate, mol/100 mol   | 16.6      | 15.1 | 17.2      | 15.5 | 1.34| ns  | ns  | ns  |
| Acetate:propionate ratio| 2.9       | 3.9  | 3.0       | 3.2  | 0.25| ns  | ns  | ns  |
| Fermentation efficiency, mol/100 mol | 72.1 | 75.0 | 73.0 | 75.5 | 1.17| ns | ns  | ns  |

°-ENZ, without enzyme; +ENZ, with enzyme; PL, particle length; ENZ, enzyme factor; INT, particle length x enzyme. ns, not significant; *, P<0.05; **, P<0.01; ***, P<0.001.
Conclusions

Fibrolytic enzyme addition has the potential to improve ruminal disappearance of diets with both short and long PL of forage. These effects were favourable enhancing the potentially disappearance of NDF and ADF of diets for growing and finishing steers. However, there were scarce effects of the enzymes on dry matter intake and ruminal fermentation. Further studies with ruminal and total tract flows of nutrients are necessary to better understand the effects of particle length of feeds on exogenous fibrolytic enzymes efficacy in ruminants.

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