Effects of exogenous fibrolytic enzymes on *in vitro* and *in sacco* degradation of diets and on growth performance of lambs

Isaac Almaraz, 1 Sergio Segundo González, 1 Juan Manuel Pinos-Rodríguez, 1 Luis Alberto Miranda 1
1Colegio de Postgraduados, Montecillo, México
2Instituto de Investigación de Zonas Desérticas, Universidad Autónoma de San Luis Potosí, México
3Departamento de Zootecnia, Universidad Autónoma Chapingo, México

**Abstract**

This study evaluated the effects of doses of exogenous fibrolytic enzymes (enzyme) on *in vitro* (IVD) and *in sacco* degradation (ISD) of dry matter (DM) and neutral detergent fibre (NDF) of diets with 70% concentrate (as DM), as well as their effects on growth performance in lambs. A gas production technique was used to determine IVD. Six ruminally cannulated lambs in a replicated 3 × 3 Latin Square were used to determine ISD. Three diets (treatments) with three levels of enzyme (0, 3 and 6 g enzyme/kg DM) were evaluated for IVD and ISD. For the growth assay, 48 lambs (17.6±2.5 kg of body weight) fed on two diets with 0 or 3 g enzyme/kg DM were used.

There were linear increases of gas production rate as enzyme level in the diets increased. At 48 h of fermentation, there were quadratic increases of IVD as enzyme level increased in the diets. A quadratic change was observed in volatile fatty acids and ammonia N as enzyme increased in the diet. At 12 h, the highest enzyme level (6 g) increased ISD of DM as compared with the control. There was a quadratic effect on the disappearance rate as enzyme level in the diet increased.

Exogenous fibrolytic enzymes improved IVD degradation and fermentation characteristics as well as ISD rate of DM, but there were not any effect of these enzymes on ISD of NDF of diets and growth performance of finishing lambs.

**Introduction**

Improving degradation of fibrous and non-fibrous carbohydrates in the rumen is important for feed utilization in ruminants. Exogenous fibrolytic enzymes have improved feed intake (Jalilvand et al., 2007; Krueger et al., 2008), which could be attributed to increased ruminal fibre digestion (Eun and Beauchemin, 2008), but the mechanism of this increment is not understood. The use of fibrolytic enzymes as feed additives to improve degradation of fibre has been studied under *in vitro*, *in sacco* and *in vivo* conditions, but the responses have been highly variable. Several factors such as enzyme doses (Colombo et al., 2007) and type of diet (Pinos-Rodriguez et al., 2008) could affect the fibrolytic activity of exogenous enzymes (Beauchemin et al., 2003). Indeed, fibrolytic enzymes increased degradation of substrates, but it depends on proportion of concentrate in the diet (Giraldo et al., 2008) and enzyme doses (Jalilvand et al., 2008). Besides, the optimal level of enzyme could depend on the diet, indicating the need to determine the optimum application rate of enzyme preparation for individual feeds (Yang et al., 1999). Therefore, the objectives of this study were to evaluate the effects of exogenous fibrolytic enzyme mixture on *in vitro* degradation, *in sacco* disappearance of a diet with 70% concentrate, as well as its effects on growth performance of lambs.

**Materials and methods**

This experiment was conducted under the supervision and approval of the Academic Committee of Instituto de Recursos Genéticos y Productividad of the Campus Montecillo, Colegio de Postgraduados, according to regulations established by the Animal Protection Law, enacted by the State of México in México. Three diets (treatments) with three levels of fibrolytic enzymes: 1) 0 g enzymes (control); 2) low level (3 g enzyme/kg DM); 3) high level (6 g enzyme/kg DM), were formulated for lambs. Diets were 70% concentrate and 30% forage (Table 1). The dry matter (DM) of feeds was determined by oven drying at 65°C to a constant weight. Samples were ground with a Wiley Mill fitted with a 1 mm screen (Arthur H. Thomas, Philadelphia, PA, USA) for chemical analysis or a 2 mm screen for *in vitro* degradation and *in sacco* ruminal disappearance determination. Hundred grams of sample were frozen at 4°C, and then analyzed for DM, nitrogen (N), acid detergent fibre (ADF), ash (AOAC, 1995), and neutral detergent fibre (NDF; Van Soest et al., 1991).

The enzyme product (Fibrozyme, Alltech Inc., Nicholasville, KY, USA) was extensively characterized by Ranilla et al. (2008), who detected that at pH 6.5 and 39°C, 1 g of enzyme preparation liberated 583 µmol of xylose per min from oat spelt xylan and 163 µmol per min of glucose from carboxymethyl-cellulose. The enzyme was a powder mixture which was mixed daily with the diet. A manual system was used to measure gas production under *in vitro* incubation at 39°C, according to Theodorou et al. (1994). The incubations were conducted in glass flasks (125 mL), sealed with butyl rubber stoppers and a screwed plastic cap, containing 90 mL of a culture medium (Malafaia et al., 1999), 10 mL of the ruminal inoculum and 500 mg of DM of experimental diets (Table 1). Rumen inoculum was obtained from lambs fed with each corresponding diet (treatment) used in the ISD trial. The samples were incubated in triplicate for each treatment and time. The gas pressure was obtained by manometric readings (0 to 1 kg/cm²), while the volume was measured by a graduated syringe (10 mL). The determinations were done at 0, 1, 2, 3, 5, 7, 9, 12, 16, 20, 24, 30, 42, 54, 66, 78, 90 and 96 h after the addition of the ruminal inoculum. Immediately after the inoculation of the ruminal inoculum, an initial reading was taken and used to standardize the pressure and discharge the gas volume in all flasks. To quantify the gas production derived from the culture medium and the ruminal inoculum, four flasks were used as a blank. The pressure and volume values were
registered and added to the values of the previous readings. Thus, the cumulative pressure and volume of the fermentation gases were obtained.

To evaluate in vitro ruminal fermentation and IVD, on 48 h of fermentation, ruminal fluid samples and DM residuals were collected from two additional glass flasks per treatment. The fluid samples were acidified with 3 M metaphosphoric acid (1:10 dilution), cooled at 4°C for 30 min, and centrifuged (25,000 x g; 4°C; 20 min). Supernatants were removed and frozen. On supernatants, volatile fatty acid (VFA; Erwin et al., 1961) with a gas chromatograph (Claurus 500, Perkin Elmer), and ammonia-N concentrations (McCullough, 1967) with a UV-VIS spectrophotometer (630 nm, CARY I-E, VARIAN), were determined. The solid residues were filtered (Whatman 541). During filtration, in order to minimize the microbial matter attached to residues, the residues were rinsed with acetone until the became clear. Filters and residues were dried for 24 h at 90°C and weighed after cooling in desiccators.

Linear regression analysis was performed with the pressure and volume data and regression coefficients were calculated. The volume of gas corrected by the pressure related to the inoculated DM was calculated according to Theodorou et al. (1994). The DM cumulative gas production profiles were evaluated using the logistic model as reported by Malafaia et al. (1999): \( V(t) = V_F / (1 + \exp(2+4(L-t))) \), where \( V \) represent the total gas produced from the digested fraction at time \( t \), its respective gas production rate \( R \) and the duration of the initial gas volume \( L \).

In vitro incubation times were used to fit non-linear regression models using the ‘NLIN’ procedure (SAS, 1999). The experimental design for this study was a completely randomized with three treatments; triplicates (glass flasks) were included to provide the error term and the GLM procedure (SAS, 1999) was used. In an in situ trial, six Criollo sheep (3.5 years old; 53±6.8 kg body weight) fitted with ruminal cannulas (Bar Diamond Inc., Parma, ID) were used in a double 3x3 Latin square design balanced for residual effects. Lambs were housed in individual metabolic pens in a 48 h period varied from 1 to 96 h, as wells as values for IVD, VFA, and ammonia N at 48 h fermentation are shown in Table 2. Enzyme did not affect parameters of gas production kinetic of DM at 0 to 96 h of fermentation, as wells as values for IVD, VFA, and ammonia N at 48 h fermentation are shown in Table 2. Enzyme did not affect

### Results

Ingredients and chemical composition of experimental diets are shown in Table 1. Parameters of gas production kinetic of DM at 0 to 96 h of fermentation, as wells as values for IVD, VFA, and ammonia N at 48 h fermentation are shown in Table 2. Enzyme did not affect

| Table 1. Ingredients and chemical composition of experimental diets. |
|-------------------------------------------------|
| Ingredients, % DM | Control (0 g) | Enzyme (g/kg DM) | Low (3 g) | High (6 g) |
|-------------------|--------------|-----------------|----------|-----------|
| Corn grain, ground | 30.0 | 30.0 | 30.0 |
| Sorghum grain, ground | 28.1 | 27.7 | 27.4 |
| Soybean meal, 44% CP | 10.9 | 10.9 | 10.9 |
| Alfalfa hay | 15.0 | 15.0 | 15.0 |
| Corn stover | 15.0 | 15.0 | 15.0 |
| Mineral premix* | 1.0 | 1.0 | 1.0 |
| Enzyme | 0.3 | 0.3 | 0.6 |

* DM base: selenium 10 mg/kg; potassium 215 mg/kg; iron 50 mg/kg; cobalt 20 mg/kg; zinc 50 mg/kg; manganese 1600 mg/kg; copper 300 mg/kg; iodine 70 mg/kg; calcium 220 mg/kg; phosphorus 260 mg/kg; sulphur 30 mg/kg; salt 950 g/kg. Fibrelzyme, Alltech Inc., Nicholasville, KY, USA.

The growth assay was analyzed as a randomized complete block design with pens as blocking factors. Because interactions of treatment \( x \) period were not significant, ADG, DMI, and feed conversion data were averaged (84 days).

Those data were analyzed using Proc Mixed (SAS, 1999). The model included block (random, 15 df) and enzyme level (fixed, 2 df), and period (fixed, 3 df). The effect of enzyme on initial BW, final BW and total gain were evaluated using the same model, except those cases in which an average value was used. The co-variance structure that resulted in the lowest Akaike’s information was AR(1), in all studies (in vitro, in sacco and in vivo), polynomial effects (linear and quadratic) were used to evaluate the effects of increasing exogenous fibrolytic enzymes in the diets.
total gas produced (V). There were linear increases for duration of initial duration of initial gas volume (L) and gas production rate (R), as the enzyme level was increased in the diet.

At 48 h fermentation, a quadratic increment of IVD was detected as enzyme level in the diet increased. At the same fermentation time, enzymes did not affect total VFA and acetate concentrations (mmol/L), but propionate and butyrate were affected quadratically as enzyme level increased in the diets. Thus, the highest concentration of propionate was with the low level (3 g) of enzyme, and the highest concentration of butyrate was with control diet (0 g enzyme). As enzyme level in the diet increased, there was a linear reduction on in vitro ammonia N concentrations.

Enzymes did not affect ISD at 3, 6, 24 and 48 h of incubation, but at 12 h 6 g enzyme/kg DM increased ISD as compared to 0 and 3 g enzyme (Figure 1). Kinetics of ISD for both, DM and NDF, are shown in Table 3. For DM, enzymes did not affect its soluble (a), potential disappearance (b) and total disappearance (a + b); however, a quadratic increase of disappearance rate was found as the enzyme in the diet increased. For NDF, enzymes did not affect the parameter of ISD kinetics.

Initial and final BW, total gain, ADG, DMI, and feed conversion (DMI/ADG) are shown in Table 4. Enzymes (3 g/kg DM) did not have any effects on these variables evaluated in lambs fed a 70% concentrate diet.

### Discussion

Level of enzyme did not affect total gas production (GP), that is, total fermentable material was not increased. Colombatto et al. (2003b) and Jalilvand et al. (2008) evaluated two levels of two enzyme products on GP and concluded that final GP values of forages were not increased by enzyme addition. The fitted GP data showed that increasing enzyme levels in the diets caused a linear increase on gas production rates. Positive responses to enzyme addition level in the rate of GP have been reported (Colombatto et al., 2003a). Our findings suggest that the enzyme was able to degrade complex substrates to simpler ones, allowing a faster ruminal colonization and fermentation, as reported by Colombatto et al. (2003a). The lack of effects on final GP suggests that the substrates degraded by the enzymes, would have been degraded in the medium anyway, albeit at a later time (Colombatto et al., 2007). In contrast, the increments

---

### Table 2. Effect of fibrolytic enzymes on kinetics of ruminal in vitro gas production of dry matter fermentation.

| Enzyme (g/kg DM) | Control (0 g) | Low (3 g) | High (6 g) | SEM | Polynomial |
|-----------------|---------------|-----------|------------|-----|------------|
| V, mL/100 mg DM|               |           |            |     |            |
| L, h            | 0.05          | 0.19      | 0.48       | 0.12| Linear     |
| R, %/h          | 0.039         | 0.043     | 0.047      | 0.001| Linear     |
| At 48 h fermentation° |          |           |            |     |            |
| Degradation, %  | 73.6          | 73.9      | 76.5       | 0.44| Quadratic  |
| Acetate, %      | 26.7          | 26.9      | 27.5       | 2.81| ns         |
| Propionate, %   | 11.1          | 12.5      | 12.1       | 0.62| Quadratic  |
| Butyrate, %     | 8.7           | 7.6       | 7.5        | 0.40| Quadratic  |
| Total VFA, %    | 46.5          | 47.0      | 47.1       | 3.58| ns         |
| A:P ratio       | 2.4           | 2.2       | 2.3        | 0.18| ns         |
| Ammonia, N mg/L | 250.2         | 244.8     | 221.9      | 23.22| Linear     |

*V, total gas produced; L, initial duration of initial gas volume; R, gas production rate.°A:P, acetate propionate ratio. SEM, standard error of the mean.

### Table 3. Effect of fibrolytic enzymes on kinetics of ruminal in sacco disappearance.

| Enzyme (g/kg DM) | Control (0 g) | Low (3 g) | High (6 g) | SEM | Polynomial |
|-----------------|---------------|-----------|------------|-----|------------|
| Dry matter, %   |               |           |            |     |            |
| Soluble fraction a | 22.4        | 21.9      | 22.3       | 1.82| ns         |
| Potential disappearance b | 51.3      | 51.0      | 52.0       | 3.29| ns         |
| Total disappearance a + b | 73.7      | 72.9      | 74.3       | 3.97| ns         |
| Disappearance rate c, %/h | 2.9       | 3.0       | 3.3        | 0.11| Quadratic  |
| Neutral detergent fibre, % |           |           |            |     |            |
| Potential disappearance b | 44.1      | 43.6      | 44.4       | 3.34| ns         |
| Disappearance rate c, %/h | 3.6       | 3.5       | 3.7        | 0.29| ns         |

SEM, standard error of the mean.

### Table 4. Effect of fibrolytic enzymes in lambs.

| Enzyme (g/kg DM) | Control (0 g) | Enzyme (3 g) | SEM |
|-----------------|---------------|--------------|-----|
| Initial BW, kg  | 17.6          | 17.1         | 0.32|
| Final BW, kg    | 35.8          | 35.5         | 0.61|
| Total gain, g   | 18.2          | 18.4         | 0.38|
| Average daily gain, ADG, g | 216.2 | 218.6 | 2.91|
| Dry matter intake, DMI, g/d | 919.9 | 921.1 | 7.48|
| Feed conversion, DMI/ADG | 4.3 | 4.2 | 0.13|

SEM, standard error of the mean; BW, body weight.
on the lag phase brought about the enzyme, as observed in our experiment, have been discussed also in low-quality roughages (Jalilvand et al., 2008; Tang et al., 2008). Indeed, Forsberg et al. (2000) postulated that addition of an enriched polysaccharidase enzyme results on an immediate attack by microorganisms, provided that there is available carbohydrate to facilitate more rapid microbial growth and shortening the lag time.

At 48 h of fermentation, the IVD of DM was increased linearly as enzyme level increased. Indeed Tang et al. (2008) found that exogenous fibrolytic enzymes (5.0 and 7.5 g/kg) increased IVD of DM of forages. In our in vitro study, the enzyme (3 g/kg DM) increased molar proportion of propionate and decreased molar proportion of butyrate as compared the control. Changes in VFA proportions as a direct effect of adding exogenous fibrolytic enzymes have been reported, implying that these enzymes could affect microbial growth and/or shift the metabolic pathways by which specific microbes utilize substrates (Eun and Beau-chemin, 2008).

At 12 h, but nor after and later, enzymes increased ISD of DM (Figure 1). The most active period for a fibrolytic enzymes mixture appears to be during the first 12 h (Moro-eno et al., 2007). It is possible that no effects would have been observed had the incubation been extended longer, which supports the hypothesis that enzymes stimulate initial phase degradation of substrate, increasing just degradation rate, but not extension of ruminal degradation (Pinos-Rodriguez et al., 2002; Giraldo et al., 2008). Although the enzyme increased ISD rate of DM, the other kinetic parameters were not affected, results that do not agree with that reported by Pinos-Rodriguez et al. (2008) who found that the same enzyme mixture enhanced potentially disappearance fraction and its disappearance rate of diets and feeds. Effects of fibrolytic enzyme mixtures are not limited to the dietary component to which the enzymes are applied. This would explain why fibrolytic enzymes can effectively improve ISD of DM fraction, besides increasing ISD of fibre components of diets, mostly when enzymes are added to the concentrate fraction of a diet or to high-concentrate diets (Beauchemin et al., 2003). Besides, Pinos-Rodriguez et al. (2002) reported that in lambs these enzymes increased apparent digestibility of CP in addition to the NDF apparent digestibility in alfalfa hay.

Beauchemin et al. (2003) indicated that fibrolytic enzymes cause highly variable responses on intake, body weight gain, and feed efficiency in finishing beef cattle fed high-grain diets. No previous evidence was found about the effect of enzymes on BW changes and feed efficiency in lambs fed 70% concentrate diets, but our results indicate that a fibrolytic enzymes mixture did not change BW in lambs. This lack of effect on growth performance could be attributed to the fact that there was not preincubation between the enzyme and the substrate (diet). Thus, a pre-incubation period is very important (Elwakeel et al., 2007; Krueger and Adesogan, 2008) to allow, before feeding, a proper adsorption and binding of the enzyme to substrate, attachment and protection against degradation by rumen proteases (Forwood et al., 1990; Beauchemin et al., 2003) and a stable enzyme feeds complex (Kung et al., 2000), all of which apparently did not place in our study.

Conclusions

The results suggest that 6 g of exogenous fibrolytic enzymes improved IVD and ISD of DM of experimental diets, however there was not any positive effects of these enzymes on ISD of NDF of diets as well on growth performance of finishing lambs. Due to this concern, further studies under in vivo conditions are needed to evaluate the factors that can affect the enzyme action to improve fibre digestion and animal performance.

References

AOAC, 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Arlington, VA, USA.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z., 2003. Use of exogenous fibrolytic enzymes to improve feed utilization by ruminants. J. Anim. Sci. 81:37-47.

Colombatto, D., Mould, F.L., Bhat, M.K., Morgavi, D.P., Beauchemin, K.A., Owen, E., 2003a. Influence of fibrolytic enzymes on the hydrolysis and fermentation of pure cellulose and xylan by mixed ruminal microorganisms in vitro. J. Anim. Sci. 81:1040-1050.

Colombatto, D., Mould, F.L., Bhat, M.K., Owen, E., 2003b. Use of fibrolytic enzymes to improve the nutritive value of ruminant diets. A biochemical and in vitro rumen degradation assessment. Anim. Feed Sci. Tech. 107:201-209.

Colombatto, D., Mould, F.L., Bhat, M.K., Owen, E., 2007. Influence of exogenous fibrolytic enzyme level and incubation pH on the in vitro ruminal fermentation of alfalfa stems. Anim. Feed Sci. Tech. 137:150-162.

Elwakeel, E.A., Tittgemeyer, E.C., Johnson, B.J., Armendariz, C.K., Shirley, J.E., 2007. Fibrolytic enzymes to increase the nutritive value of dairy feedstuffs. J. Dairy Sci. 90:5226-5236.

Erwin, E.S., Marco, G.J., Emery, E.M., 1961. Volatile fatty acid analysis of blood and rumen fluid by gas chromatography. J. Dairy Sci. 44:1768-1776.

Eun, J.S., Beauchemin, K.A., 2008. Relationship between enzymatic activities and in vitro degradation of alfalfa hay and corn silage. Anim. Feed Sci. Tech. 14:53-67.

Forsberg, C., Forano, E., Chesson, A., 2000. Microbial adherence to the plant cell wall and enzymatic hydrolysis. In: PB. Cronje (ed.) Ruminant Physiology: Digestion, Metabolism, Growth and Reproduction. CABI Publishing, Wallingford, UK. pp 79-97.

Forwood, J.R., Sleper, D.A. Henning, J.A., 1990. Topical cellulose application effects on total escape digestibility. Agron. J. 82:909-913.

Giraldo, L.A., Tejido, M.L., Ranilla, M.I., Carro, M.D., 2008. Effects of exogenous fibrolytic enzymes on in vitro ruminal fermentation of substrates with different forage-concentrate ratios. Anim. Feed Sci. Tech. 141:306-325.

Jalilvand, G., Naserian, A., Odongo, N.E., Kebreah, E., Valizadeh, R., Effekhar Shahrodi, F., France, J., 2007. Effects of abomasal infusion of cottonseed oil and dietary enzyme supplementation on dairy goats. J. Anim. Feed Sci. 16:389-396.

Jalilvand, G., Odongo, N.E., López, S., Naserian, A., Valizadeh, R., Effekhar Shahrodi, F., Kebreah E., France, J., 2008. Effects of different levels of an enzyme mixture on in vitro gas production parameters of contrasting forages. Anim. Feed Sci. Tech. 146:289-301.

Krueger, N.A., Adesogan, A.T., 2008. Effects of different mixtures of fibrolytic enzymes on digestion and fermentation of bahiagrass hay. Anim. Feed Sci. Tech. 145:84-94.

Krueger, N.A., Adesogan, A.T., Staples, C.R., Krueger, W.K., Kim, S.C., Littell, R.C., Sollenberger, L.E., 2008. Effect of method of applying fibrolytic enzymes or ammonia to Bermudagrass hay on feed intake, digestion, and growth of beef steers. J. Anim. Sci. 86:882-889.

Kung, L.Jr., Treacher, R.J., Nauman, G.A., Smagala, A.M., Endres, K.M., Cohen, M.A., 2000. The effect of treating forages with fibrolytic enzymes on its nutritive value and lactation performance of dairy cows. J.
Dairy Sci. 83:115-122.
Malafaia, P.A.M., Filho, S.C.V, Vieira, R.A.M., 1999. Kinetic parameters of ruminal degradation estimated with a non-automated system to measure gas production. Livest. Prod. Sci. 58:65-73.
McCullough, H., 1967. The determination of ammonia in whole blood by a direct colorimetric method. Clin. Chem. Acta 17:297-304.
Moreno, R., Pinos-Rodríguez, J.M., González, S., Álvarez, G., García, J.C., Mendoza, G., Bárcena, R., 2007. Effect of exogenous fibrolytic enzymes on in vitro ruminal degradation of rations for dairy cows. Inter ciencia 32:850-853.
Pinos-Rodríguez, J.M., González, S.S., Mendoza, G., Bárcena, J.R., Cobos, M.A., Hernández, A., Ortega, M.E., 2002. Effect of exogenous fibrolytic enzyme on ruminal fermentation and digestibility of alfalfa and rye-grass hay fed to lambs. J. Anim. Sci. 80:3016-3020.
Pinos-Rodríguez, J.M., Moreno, R., González, S.S., Robinson, P.H., Mendoza, G., Álvarez, G.A., 2008. Effects of exogenous fibrolytic enzymes on ruminal fermentation and digestibility of total mixed rations fed to lambs. Anim. Feed Sci. Tech. 142:210-219.
Ranilla, M.J., Tejido, M.L., Giraldo, L.A., Tricá rico, J.M., Carro, M.D., 2008. Effects of an exogenous fibrolytic enzyme preparation on in vitro ruminal fermentation of three forages and their isolated cell walls. Anim. Feed Sci. Tech. 145:109-121.
SAS, 1999. User’s Guide. Version 6.12. SAS Institute Inc. Cary, NC, USA.
Susmel, P., Spanghero, M., Stefanon, B., 1999. Interpretation of rumen degradability of concentrate feeds with a Gompertz model. Anim. Feed Sci. Tech. 79:223-237.
Tang, S.X., Tayo, G.O., Tan, Z.L., Sun, Z.H., Shen, L.X., Zhou, C.S., Xiao, W.J., Ren, G.P., Han, X.F., Shen, S.B., 2008. Effects of yeast culture and fibrolytic enzymes supplementation on in vitro. J. Anim. Sci. 86:1164-1172.
Theodorou, M.K., Williams, B.A., Dhanoa, M.S., McAllan, A.B., France, J., 1994. A simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim. Feed Sci. Tech. 48:185-197.
Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. J. Dairy Sci. 74:3583-3597.
Yang, W.Z., Beauchemin, K.A., Rode, L.M., 1999. Effects of an enzyme feed additive on extent of digestion and milk production of lactating dairy cows. J. Dairy Sci. 82:391-403.