THE INFLUENCE OF CELLUPRACT AS100® ENZYME TREATMENT TO RICE BRAN AND COTTON SEED MEAL ON CHEMICAL COMPOSITION, ENERGY CONTENT AND *IN VITRO* DIGESTIBILITY

PENGARUH PERLAKUAN ENZIM *CELLUPRACT AS100®* PADA DEDAK PADI DAN TEPUNG BIJI KAPAS TERHADAP KOMPOSISI KIMIA, KANDUNGAN ENERGI DAN KECERNAAN *IN VITRO*

M. Bata[1,3], S.N.O. Suwandyastuti[1], S. Chakeredza[2], G. Thinggaard[3] and U. ter Meulen[3]

[1]Jenderal Soedirman University, Faculty of Animal Science, Department of Animal Nutrition, P.O. Box 10, Purwokerto, Central Java, Indonesia
[2]Africa University, Faculty of Agriculture and Natural Resources, P. O. Box 1320, Mutare, Zimbawe
[3]Institute of Animal Physiology and Animal Nutrition, Georg-August University of Goettingen, Kellnerweg 6, D-37077 Goettingen, Germany

ABSTRACT

The effect of CelluPract AS100® enzyme treatment to rice bran (RB) and cottonseed meal (CSM) on chemical composition, energy content and digestibility was evaluated in three experiments. In experiment 1, enzyme levels of 0, 1.5, 3.0 and 4.5 g kg^{-1} DM sample were used to treat RB and CSM in plastic bags for 24 hours under room temperature conditions of 26°C. Treated and untreated samples were analysed for proximate constituents. The gas test was used in the second experiment to evaluate the effect of treatment on metabolisable energy (ME) content. In the third experiment intestinal nitrogen digestibility was assessed. Enzyme treatment tended to improve the crude protein (CP) and decrease the crude fibre (CF) and nitrogen free extract (NFE) contents in both RB and CSM. The ME content was significantly ($P<0.05$) highest at 1.5 g kg^{-1} DM enzyme treatment being 7.56 and 6.16 MJ kg^{-1} DM, respectively on RB and CSM compared to the respective control values of 6.90 and 5.23 MJ kg^{-1} DM. No clear pattern emerged on intestinal nitrogen digestibility and RB and CSM averaged, respectively 72.1 and 64.6 per cent digestibility. These experiments demonstrate the beneficial effects to nutritive value of treating feeds with fibrolytic enzyme and for CelluPract AS100®, the optimum level for both feedstuffs was 1.5 g kg^{-1} DM. With the current high costs of feed and low cost of enzyme preparations, these enzymes have potential for use in ruminant feeding systems.

Key words: Rice bran, Cottonseed meal, Fibrolytic enzyme, CelluPract AS100®

ABSTRAK

Pengaruh perlakuan enzyme Cellupract AS 100® pada dedak padi kasar dan bungkil biji kapok terhadap komposisi kimia, kandungan energy dan kecernaan dievaluasi melalui tiga percobaan. Percobaan 1, taraf enzyme 0, 1,5, 3,0 dan 4,5 g/kg DM sampel diberikan pada dedak padi kasar dan bungkin biji kapok dalam plastik yang diinkubasi selama 24 jam pada suhu 26 °C. Analisa proksimat dilakukan terhadap baik kontrol maupun perlakuan. Gas Test digunakan pada percobaan 2 untuk mengevaluasi pengaruh enzyme terhadap kandungan metabolizable energy (ME). Pada percobaan 3, kecernaan nitrogen dalam intestinum dievaluasi. Perlakuan enzyme cenderung untuk meningkatkan kandungan protein dan menurunkan kandungan serat kasar dan bahan extrak tanpa nitrogen baik pada dedak maupun bungkil biji kapok. Kandungan ME tertinggi ($P<0,05$) pada taraf enzim 1,5 g/kg DM adalah 7.56 and 6.16 MJ kg^{-1} DM secara berturut-turut untuk dedak padi kasar dan bungkil biji kapok dibanding dengan kontrol 6.90 and 5.23 MJ kg^{-1} DM. Tidak jelas pengaruh perlakuan terhadap pola kecernaan nitrogen dalam intestinum dan rataan kecernaannya adalah 72.1 and 64.6 persen untuk dedak dan bungkil biji kapok. Percobaan ini menunjukkan keuntungan menggunakan enzim untuk meningkatkan nilai nutrisi dan untuk enzim CelluPract AS100®, taraf optimum untuk kedua bahan pakan tersebut adalah 1,5 g/kg DM. Dengan harga pakan yang tinggi dan biaya penyediaan enzim yang rendah, enzim ini mempunyai potensial untuk digunakan pada sistem pemberian pakan untuk ruminansia.

Kata kunci: Dedak padi kasar, bungkil biji kapok, enzim cellulase komplek, CelluPract AS 100®.

INTRODUCTION

The major energy and protein supplements used in ruminant livestock production in Indonesia are rice bran (RB) and cottonseed meal (CSM), respectively. Due to the method of processing, these feedstuffs are high in fibre content. Their fermentation in the rumen leads to high acetate production relative to other volatile fatty acids. In tropical and subtropical environments where temperature humidity index is high, if excess acetogenic substrates are not utilised for tissue synthesis, then acetate must be dissipated as heat. If the animal is able to oxidise the acetogenic substrate but cannot dissipate the heat generated because environmental temperature and humidity are high, then it could allow its body temperature to increase to some extent, but eventually it must reduce its feed intake (Blaxter 1989). This ultimately leads to lower animal productivity.

Fibrolytic enzyme supplementation to forage based diets have been used to improve the feed value for ruminant animals (Rode *et al.* 1999, Kung *et al.* 2000). The beneficial effects of fibrolytic enzymes in ruminant diets appear to be a result of, in part, improvement in feed digestibility (Yang *et al.* 2000) leading to increased digestible energy intake. However, when viewed across a variety of enzyme products and experimental conditions, the respective response to feed enzymes by ruminants has been variable (Beauchemin *et al.* 2003). Variable results obtained in literature are largely due to substrate specificity, time required for enzymes to interact with substrate and pH, activities and characteristics of the enzymes supplied, temperature of feed during treatment and method of providing the enzyme to the animal. There is currently a dearth of information regarding effect of specific fibrolytic enzymes on nutritive value improvement of given feedstuffs particularly with regard to ruminant animals.

This study was set up with the objectives of evaluating the influence of CelluPract AS100® enzyme treatment to RB and CSM on changes in nutrient profile, energy content, and intestinal nitrogen digestibility.

MATERIALS AND METHODS

Three series of experiments were carried out at Jenderal Soedirman University, Central Java, Indonesia. The influence of enzyme treatment on RB and CSM nutrient changes was evaluated in experiment one. Changes in metabolisable energy (ME) content of the fermented feed using the Menke *in vitro* gas technique was assessed in experiment two while in experiment three, intestinal nitrogen digestibility of the fermented feeds was evaluated.

Enzyme treatment

Enzyme Preparations

CelluPract AS100® enzyme, a cellulase enzyme consisting of cellulase, β-glucosidase, carboxymethylcellulase and hemicellulase, was obtained from Biopract GmbH, Berlin, Germany. The enzyme constituted the treatments and was used at levels of: 0, 1.5, 3.0 and 4.5 g kg^{-1} DM of feed sample. The enzyme was dissolved in distilled water at 100 g L^{-1} according to Krause *et al.* (1998) before mixing it with the feed sample.

Feed sample preparation

Rice bran and CSM were obtained from a local factory in Indonesia. They were ground with a blender (Super Grinder HL 1641, Philips Pvt. Ltd.) to pass through a 1 mm screen size before enzyme addition.

Experimental Procedure

Sample incubation

Fifty-gram feed samples of RB and CSM were weighed into individual polythene bags measuring 30 x 20 cm. There were four bags per enzyme treatment. The enzyme preparation was then thoroughly mixed with the feed sample in a blender according to treatment and incubated at room temperature under aerobic conditions for 24 hours. At the end of the incubation, samples were pooled together by treatment and analysed for proximate constituents.

In vitro gas test assessment

The Menke *in vitro* gas test (Menke *et al.* 1979) was conducted on the treated RB and CSM samples. The same treatments used in the enzyme fermentation study were maintained.

Rumen liquor

Rumen liquor was obtained from a rumen fistulated Peranakan Ongole cow. The cow had been maintained on a Napier grass (*Pennisetum purpureum*): concentrate feed offered at 70:30 w/w, respectively. The concentrate was offered at 0700 hours while the Napier grass was offered at 1100 and 1600 hours. About 1.5 litres of rumen liquor was collected before feeding. The liquor was prepared by straining digesta contents through two layers of muslin cloth and put into a pre-warmed thermos flask. Carbon dioxide was used to maintain anaerobic conditions.

Media preparation

A buffer medium consisting of 400 ml distilled water, 0.1 ml micromineral solution, 200 ml rumen buffer solution, 200 ml macromineral solution, 1.0 ml resazurin and 40 ml reducing solution was prepared immediately before rumen liquor collection and kept under anaerobic conditions (using CO_2) in a water bath maintained at 39°C. One part of the rumen liquor was

mixed with two parts of the medium to have a rumen liquor buffer medium (RL-M) which was maintained under anaerobic conditions at 39°C until sample incubation.

Sample Incubation

Twenty-one graduated glass syringes were used. The syringes were of 36 mm external diameter, 200 mm long with a calibrated volume of 100 ml. The outlets were fitted with silicon tubes (50 mm in length and 5 mm internal diameter) and clips were used to close the tubes. Three tubes were allocated to each treatment.

Approximately 230 mg of sample from the enzyme fermentation study were weighed and placed into the glass syringes. Syringes with standard hay and concentrate obtained from Hohenheim University, Germany, together with a blank were also treated as for the other syringes. Each of the syringes was then filled with 30 ml of RL-M using an automatic pipette and incubated for 24 hours at 39°C. The changes in gas volume were calculated. The gas production was used to calculate the ME content according to the equation of Close and Menke (1986): ME (MJ kg^{-1} DM) = 1.06 + 0.157 Gb + 0.0084 CP + 0.022 EE - 0.0081 ash, where Gb is gas volume measured after 24 hours in ml with CP (crude protein), EE (ether extract) and ash contents being in percentage.

Intestinal nitrogen digestibility

Animals and feeding

A rumen fistulated Peranakan Ongole cow was used. The cow had been maintained on the same feeding regime as described under *in vitro* gas test assessment.

Experimental Procedure

Nylon bag samples

Degradation of enzyme treated RB and CSM samples were obtained by incubating the samples in nylon bags suspended in the rumen of the cannulated cow according to the technique described by Ørskov *et al.* (1980). The bags (170 x 90 mm; 47 µm pore size) were made of nylon fabric. They were sewn with a double row of stitching with rounded corners to allow for easy removal of particulate material. Air dry feed samples from enzyme treatment were weighed, 1 g into the bags. Three bags were weighed per treatment and these were suspended by a 40 cm nylon cord. The bags were soaked in water for 20 minutes and then inserted into the rumen. All the bags were removed from the rumen after 16 hours and washed under running tap water until the water gently squeezed from them was clear. After washing, the bags and their contents were dried at 55°C in a forced draught oven for 48 hours. The procedure was repeated twice. The samples were stored for nitrogen (N) analysis and incubation in HCL-pepsin solution.

Determination of intestinal nitrogen digestibility

For each 15 mg of residual nitrogen, residues of nylon bag samples were incubated with 10 ml of 0.1 N HCl solution containing 1g L^{-1} of pepsin and then incubated at 38°C for 24 hours in a shaking water bath.

At the end of the incubation period, 3 ml of 100 per cent (w/v) solution of trichloroacetic acid (TCA) were added to the tubes to stop enzymatic action and to precipitate undigested protein. Samples were centrifuged at 10 000 g for 15 minutes and the supernatant was collected for nitrogen analysis.

Calculations

Intestinal N digestibility was determined following the method of Calsamiglia *et al.* (1995) as:

$$\text{Intestinal N digestibility\%} = \frac{\text{TCA soluble N}}{\text{Total N after 16h degradation}} \times 100$$

Chemical analyses

Original samples of RB and CSM and also after enzyme treatment were analysed for DM, CP, crude fibre (CF), EE and ash. Nitrogen free extract (NFE) was obtained by difference. Samples from the 16 h nylon bag degradation were analysed for DM and N content. Supernatant samples after pepsin enzyme digestion and centrifugation were analysed for nitrogen content. These methods were carried out according to AOAC (1990) procedures.

Statistical Analysis

All data were subjected to analysis of variance using the general linear models procedure of the statistical analysis system (SAS 1996). Treatment means were compared using the Duncan's multiple range test.

RESULTS AND DISCUSSION

The certified enzyme activity data are shown in Table 1. CelluPract AS100® had the following enzym: xylanase, ß-glucanase, carboxymethylcellulase and hemicellulase.

The average laboratory temperature over the 24 hour incubation period was 26°C. The proximate composition of RB and CSM without enzyme treatment and following enzyme treatment is shown in Table 2. Since samples were all pooled together by treatment for chemical analyses only mean values are presented and no statistical analyses were done on them. The tendency was to have an increase in CP content and a decrease in CF and NFE contents in both RB and CSM. Ether extract and ash content remained fairly constant.

Table 1. Certified activity of CelluPract AS100 in U g^{-1} ± SD.

Enzyme	Certified enzyme activity (Biopract, GmbH), Berlin, Germany
Hemicellulase	96 ± 4
Carboxymethylcellulase	2 800 ± 8
ß-glucanase	4 400 ± 3
Xylanase	5 600 ± 4

Table 2. Proximate analysis (%) of rice bran and cottonseed meal treated with different levels of CelluPract AS100 enzyme

Enzyme level g kg^{-1} DM sample	Dry Matter (DM)	Percentage DM				
		Crude Protein	Ether Extract	Crude Fibre	Ash	Nitrogen Free extract
Rice Bran						
0	76.08	10.56	13.59	16.77	11.09	47.49
1.5	75.77	12.00	12.06	16.60	11.54	47.80
3.0	75.48	12.27	12.19	16.37	12.29	46.88
4.5	75.07	12.06	12.29	16.23	11.96	46.96
Cottonseed meal						
0	79.99	28.99	23.59	27.16	9.40	10.86
1.5	80.81	33.05	23.92	26.36	8.97	8.11
3.0	80.29	33.62	23.33	24.28	9.07	9.07
4.5	82.76	33.20	23.40	23.09	9.09	10.28

Gas production and calculated ME contents in both RB and CSM are shown in Table 3. Enzyme addition increased gas production. However, the increase was significant ($P<0.05$) only at 1.5 g kg^{-1} DM enzyme treatment compared to the control. Beyond 1.5 g kg^{-1} DM enzyme treatment, gas production declined. This trend was the same for ME changes.

Changes in gas production and ME contents in CSM also significantly ($P<0.05$) peaked at 1.5 g kg^{-1} DM enzyme treatment compared to the control. Beyond 1.5 g kg^{-1} DM enzyme treatment both gas production and ME content declined.

Data for intestinal nitrogen digestibility for both RB and CSM are shown in Table 4. No clear pattern emerged although for both RB and CSM, highest numerical values for nitrogen digestibility were obtained at 4.5 g kg^{-1} DM enzyme treatment. On average intestinal N digestibility was 72.1 and 64.6 per cent for RB and CSM, respectively.

Fibrolytic enzyme treatment has been observed to decrease neutral detergent fibre (NDF) fraction of diet and increase reducing sugars and CP contents. This

Table 3. Mean total gas production and metabolisable energy content of rice bran and cottonseed meal treated with different levels of enzyme

Parameter	Enzyme level g kg^{-1} dry matter				SED
	0	1.5	3.0	4.5	
Rice bran					
Gas production (ml)	33.33^{a}	39.70^{b}	35.43^{ab}	36.22^{ab}	4.903
ME* (MJ kg^{-1} DM)	6.90^{a}	7.56^{b}	6.85^{a}	7.01^{a}	0.038
Cottonseed meal					
Gas production (ml)	23.21^{a}	27.91^{b}	25.46^{ab}	24.65^{a}	0.159
ME (MJ kg^{-1} DM)	5.23^{a}	6.16^{b}	5.75^{c}	5.60^{ac}	0.037

ME = Metabolisable energy.
SED = standard error of difference between means.
^{abc}Means within the same row not having at least a common superscript are significantly different ($P<0.05$).
*Calculated from the relation of Close and Menke (1986) of: ME (MJ kg^{-1} DM) = 1.06 + 0.157 Gb + 0.0084 CP + 0.022 EE - 0.0081 ash, where Gb is gas volume measured after 24 hours in ml; CP, EE and ash contents are in percentage. CP and EE are Crude protein and ether extract, respectively.

Table 4. Intestinal nitrogen digestibility (%) of rice bran and cottonseed meal following treatment with different levels of enzyme

Feedstuff	Enzyme level g kg^{-1} dry matter				SED
	0	1.5	3.0	4.5	
Rice bran	69.24^{a}	70.18^{a}	73.13^{b}	75.80^{c}	0.892
Cottonseed meal	60.75^{ab}	60.04^{a}	66.65^{ab}	70.80^{b}	2.457

SED = standard error of difference between means.
^{abc}Means in the same row not having at least a common superscript are significantly different ($P<0.05$).

improved nutrient profile has led to higher ME content and overall improved nutrient digestibility with resultant improved animal performance on animals receiving diets treated with enzymes.

In the current study, CF was reduced and CP was increased with enzyme treatment in both RB and CSM. These observations are in agreement with work of Hristov *et al.* (1998) who reported that fibrolytic enzyme treatment of a total mixed ration of rolled barley grain, corn silage and soyabean meal led to decreased NDF content and increased reducing sugars and CP content. Krause *et al.* (1998) also reported that enzyme treatment of concentrate and barley silage and concentrate and barley straw tended to increase CP and significantly decrease acid detergent fibre (ADF) and NDF contents.

The main components of CF in proximate analysis are cellulose, hemicellulose and lignin, the major structural polysaccharides in plants. Fibrolytic enzymes containing mainly cellulase, ß-glucanase and xylanase can hydrolyse cellulose and hemicellulose to glucose. The optimum level of CelluPract AS100® used in this study for both RB and CSM was 1.5 g kg^{-1} DM feed. Beyond this level, the effect was negative. In other studies Bhat and Hazlewood (2001) found that cellulases and xylanases were inhibited by the presence of high concentrations of their hydrolysis products. ß-glucosidases were inhibited by glucose and other mono- and disaccharides such as xylose, fucose, galactose, maltose, lactose and meliobiose (Bhat *et al.* 1993). While hydrolysis products were not assessed in the current study, we can surmise that at 3.0 and 4.5 g kg^{-1} DM enzyme treatment, accumulation of hydrolysis end-products could have negatively inhibited further enzyme activity. With the enzyme treatment having lowered the CF content and increased the CP content through fibrolytic enzyme activity the nutritive value of RB and CSM in terms of ME should have been increased.

In vitro gas test data in this study showed that total gas production and ME content were also maximised at enzyme level of 1.5 g kg^{-1} DM in both RB and CSM. Increase in gas production following enzyme treatment has also been noted before. Beauvink (1993) combined cellulase (C) from *Trichoderma viride*, hemicellulase (H) from *Aspergillus niger* and rapidase, a pectinase (P) from *Aspergillus niger* ending with the treatments HC, HP, CP and HCP which the author used to treat ryegrass silage. Only HP enzyme was the same as the control in terms of gas production kinetics and chemical composition. The author concluded that cellulase component was necessary to establish changes in both gas production and chemical composition. In contrast, Iwaasa *et al.* (1998) reported that gas production increased after alfalfa forage was treated with fibrolytic enzyme containing mainly pectinase. This suggests that the combination of enzymes depends largely on the feed to be treated. There is need to match the enzyme product to the forage being treated.

Beyond 1.5 g kg^{-1} DM enzyme treatment of RB and CSM, there was no further increase in gas production. This could be related to the mode of microbial attachment to and fermentation of feed substrate. Treacher and Hunt (1996) have suggested three possible explanations for reduced animal performance with excessive addition of enzymes related to activity of microbes. Firstly it is possible that high doses of enzymes reduce the amount of NDF available for fermentation by rumen microorganisms which in turn reduces microbial protein and VFA production. Secondly, the presence of bound enzymes restricts the access of microbes to feed. Third, enzymes release antinutritional by-products like phenols which inhibit microbial growth.

Nsereko *et al.* (2002) reported a quadratic response in total bacterial numbers in ruminal fluid with increasing levels of an enzyme product from *Trichoderma longibrachiatum* added to a dairy cow diet. These authors suggested that application of low levels of enzyme to ruminant feeds caused some beneficial disruption of the surface structure of the feed either before or after ingestion. When excess enzyme was applied, the beneficial disruption of the feed surface structure may have diminished because the excess exogenous enzyme attached to the feed may have restricted microbial attachment and limited digestion of feed. It appears that more work needs to be carried out to elucidate the mode of action of the negative effect of high levels of enzyme treatment on feed fermentation by rumen microbes.

Intestinal nitrogen digestibility, showed highest numerical values at 4.5 g kg^{-1} DM enzyme treatment. There was no clear enzyme effect though. This might imply that benefits of fibrolytic enzyme treatment largely occur at the ruminal level.

CONCLUSIONS

This study demonstrates the beneficial effects to nutritive value of treating high fibre feeds with fibrolytic enzymes and 1.5 g kg^{-1} DM CelluPract AS100® enzyme treatment was optimal for RB and CSM treatment in terms of chemical composition changes and fermentation under *in vitro* conditions. With the current high feed costs and lower costs of enzyme manufacturing, enzymes have potential for use in ruminant feeding systems.

REFERENCES

AOAC, 1990. Official methods of analysis, 13th Edition. Association of Official Analytical Chemists, Washington, DC.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z., 2003: Use of exogenous fibrolytic enzymes to improve feed utilisation by ruminants. J. Anim. Sci. 81 (E. Suppl. 2), E37.

Beauvink, J.M.W., 1993: Measuring and modelling *in vitro* gas production kinetics to evaluate ruminal fermentation of feedstuffs. DLO-Instituut voor veevoedingsornderzoek (IVVO-DLO). Lelystad.

Bhat, M.K., Gaikwad, J.S., Maheshwari, R., 1993: Purification and characterisation of extracellular ß-glucosidase from the thermophilic fungus *Sporotrichum thermophile* and its influence on cellulase activity. J. Gen. Microbiol. 39, 2825.

Bhat, M.K., Hazlewood, G.P., 2001: Enzymology and other characteristics of cellulases and xylanases. Page 11 in Enzymes in Farm Animal Nutrition. M. Bedford and G. Partridge ed. CABI, Publishing, Oxon, UK.

Blaxter, K.L., 1989: Energy Metabolism in Animals and Man. Cambridge University Press, 336 pp.

Calsamiglia, S., Stern, M.D., Firkins, J.L., 1995: Effects of protein source on nitrogen metabolism in continuous culture and intestinal digestion *in vitro*. J. Anim. Sci. 73, 1819.

Close, W.H., Menke, K.H., 1986: Selected topics in Animal Nutrition. A manual prepared for the 3rd Hohenheim course on animal nutrition in the tropics and semi-tropics. 2nd edition. University of Hohenheim, Stuttgart, Germany. DSE, Feldafing, Germany.

Hristov, A.N., McAllister, T.A., Cheng, K.-J., 1998: Stability of exogenous polysaccharide-degrading enzyme in the rumen. Anim. Feed Sci. Technol. 76, 161.

Iwaasa A.D., Rode L.M., Beauchemin K.A., Eivemark, S., 1998: Cumulative gas production of alfalfa forage treated with different cell wall degrading enzyme. J. Dairy Sci. 81 (Suppl.), 291 (Abstr.).

Krause, M., Beauchemin, K.A., Rode, L.M., Farr B.I., Norgaard, P., 1998: Fibrolytic enzyme treatment of barley grain and source of forage in high grain diets fed to growing cattle. J. Anim. Sci. 96, 1010.

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.

Menke, K.H., Raab, L., Salewiski, A., Steingass, H., Fritz, D., Schneider, W., 1979: The estimation of digestibility and metabolisable energy content of ruminant feedstuffs from the gas production when they are incubated with rumen liquor. J. Agric. Sci. Camb. 93, 217.

Nsereko, V.L., Beauchemin, K.A., Morgavi, D.P., Rode, L.M., Furtado, A.F., McAllister, T.A., Iwaasa, A.D., Yang, W.Z., Wang, Y., 2002: Effect of fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. Can. J. Microbiol. 48, 14.

Ørskov, E.R., Hovell, F.D. Deb., Mould, F.L., 1980: The nylon bag technique for nutritional studies in ruminants. Trop. Anim. Prod. 5, 195.

Rode, L.M., Yang, W.Z., Beauchemin, K.A., 1999: Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. 82, 2121.

SAS, 1996. SAS/STAT User's Guide (Release 6.11) SAS Inst. Inc., Cary, North Carolina.

Treacher, R.J., Hunt, C.W., 1996: Recent developments in feed enzymes for ruminants. Pacific Northwest Nutrition Conference.

Yang, W.Z., Beauchemin, K.A., Rode, L.M., 2000: A comparison of methods of adding fibrolytic enzymes to lactating cow diets. J. Dairy Sci. 83, 2512.