EFFECTS OF EXOGENOUS FIBROLYTIC ENZYMES ON *IN VITRO* FERMENTATION KINETICS OF FORAGE AND MIXED FEED SUBSTRATES

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Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature ……. Date: 04-03-2008

Abstract

Title : Effects of exogenous fibrolytic enzymes on *in vitro* fermentation kinetics of forage and mixed feed substrates

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Two *in vitro* experiments were conducted to evaluate the effect of exogenous fibrolytic enzyme application on dry matter (DM) and neutral detergent fibre (NDF) degradation and gas production (GP) of mature forages and forage-concentrate mixtures. The forages used in the first experiment were lucerne hay (LH), oat hay (OH) and wheat straw (WS). The same forages were used in the second experiment, but they were mixed with a concentrate feed to make three mixtures consisting of 80% (HC), 50% (MC) or 20% (LC) concentrate. The extracellular enzyme fraction (supernatant) of a fungal strain, ABO 374, was used as feed additive. The supernatant was used in a fresh (SU-ABO374) or lyophilized (CSIR-ABO374) form, the latter being reconstituted with water immediately before application. The liquid supernatants were applied to the incubation medium and not directly to the substrate, at a rate equivalent to 7.5 ml/kg feed DM. In the control treatments of both experiments, water was used instead of the liquid supernatants. For the DM and NDF degradability trials in both experiments, 500 mg forage samples were weighed into 50 x 50 mm dacron bags which were incubated anaerobically at 39ºC in 1.4L of a rumen liquid inoculated buffered medium in 2L fermentation jars. Bags from all treatments were removed after 2, 4, 8, 12, 24, 48, 72 and 96 h of incubation. For the gas production determinations, 500 mg of the respective substrate samples were weighed into 120 ml glass vials which were incubated for 96 h in 40 ml inoculated medium to which 0.5 ml of the respective enzyme solutions were added. Gas pressure was recorded manually with a digital pressure gauge after 2, 4, 8, 12, 24, 48, 72 and 96 h and pressure was converted to volume with a predetermined regression. The 96 h substrate residues were washed, dried, weighed and analyzed for NDF and OM. In both experiments the substrates

differed in terms of DM and NDF degradability and gas production rates, but the enzyme treatments had no effect. The lack of response to enzyme application was ascribed to a number of factors, including the fact that enzyme application was into the incubation medium and not directly onto the substrates and also that no significant pre-incubation interaction time was allowed. The same preparations gave positive results in previous trials where they were applied directly onto the substrates and where a pre-incubation interaction time of 16 hours was allowed.

(*Key words*: Exogenous enzymes, forages, concentrate based diets, DM and NDF degradation, gas production)

Uittrekksel

Titel : Die invloed van eksogene fibrolitiese ensieme op *in vitro* fermentasiekinetika van ruvoer- en gemengde voersubstrate

Naam : Thembekile Feonah Baloyi

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Twee *in vitro*-experimente is uitgevoer om die invloed van eksogene fibrolitiese ensieme op droëmateriaal (DM) en neutraal-onoplosbare vesel (NDF) degradering en gasproduksie (GP) van volwasse ruvoersubstrate en ruvoer-kragvoermengsels te bepaal. Ruvoere in die eerste eksperiment was lusernhooi (LH), hawerhooi (HH) en koringstrooi (KS). Dieselfde ruvoere is in die tweede eksperiment gebruik, maar hulle is met ‘n kragvoer gemeng om drie mengsels te maak, bestaande uit 80% (HK), 50% (MK) of 20% (LK) kragvoer. Die ekstrasellulêre ensiemfraksie (supernatant) van ‘n fungiale stam, ABO 374, is as ‘n voertoedieningsmiddel gebruik. Die supernatant is is in ‘n vars (SU-ABO374) of gevriesdroogde (WNNR-ABO374) vorm gebruik, waar laasgenoemde onmiddellik voor toediening gerekonstitueer is. Die vloeistof-supernatante is nie direk op die substrate gevoeg nie, maar tot die inkubasiemedium gevoeg, teen ‘n hoeveelheid ekwivalent aan 7.5 ml/kg voer DM. In die kontrolebehandeling van beide eksperimente, is water in plaas van die vloeistofsupernatante gebruik. Vir die DM- en NDF-degraderingsproewe in beide eksperimente, is 500 mg van die onderskeie ruvoere in 50 x 50 mm dacronsakkies geweeg wat anaerobies by 39ºC geïnkubeer is in 1.4L van ‘n rumenvloeistof-geïnokkuleerde medium in 2L fermentasieflesse. Vir alle behandelings is sakkies na 2, 4, 8, 12, 24, 48, 72 en 96 h inkubasie verwyder. Vir gasproduksiebepalings is 500 mg van die onderskeie substraatmonsters in 120 ml glasbotteltjies geweeg en vir 96 h in 40 ml geïnokkuleerde medium geïnkubeer waarin 0.5 ml van die onderskeie ensiemoplossings gevoeg is. Gasdruk is na 2, 4, 8, 12, 24, 48, 72 en 96 h bepaal met behulp van ‘n digitale drukmeter en druk is met behulp van ‘n voorafbepaalde regressie na volume omgeskakel. Die 96 h substraatresidue is gewas, gedroog, geweeg en ontleed vir NDF en OM. In beide

eksperimente het die substrate verskil ten opsigte van DM- en NDF-degradeerbaarheid en gasproduksietempo's, maar die ensiembehandelings het geen invloed gehad nie. Die gebrek aan respons is aan verskeie faktore toegeskryf, insluitend die feit dat ensiemtoediening in die inkubasiemedium toegedien is en nie direk op die substrate nie, asook die feit dat daar nie 'n noemenswaardige pre-inkubasie interaksietyd toegalaat is nie. Dieselfde ensiempreparate het positiewe resultate gelewer in vorige proewe waar dit direk op die substraat toegedien is en waar 'n pre-inkubasie interaksietyd van 16 ure toegelaat is.

(*Sleutelwoorde:* Eksogene ensieme, ruvoere, kragvoerdiëte, DM- en NDF-degradering, gasproduksie)

Dedication

This thesis is dedicated to my GOD who watched over me from a distance, my family and to my late father and mother (Songi) who never had a chance to see my success.

I can do all things through Christ who strengthens me

Philippians: 4 vs. 13

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CHAPTER 1

General Introduction

Ruminant animals utilize grass and leguminous forages as sources of nutrients via a symbiotic relationship with rumen microbes (Hungate, 1966). Forages are an important source of fibre which contribute to proper digestion and enhances salivation and rumen buffering (Mertens, 1997), subsequently improving degradation of cell wall material by ruminants. However, intake and digestibility of plant cell wall material by ruminants is often limited by availability of good quality forages (Van Soest, 1994), thus affecting productivity.

A common problem facing ruminant animal production in South Africa is the general lack of providing a constant supply of good quality forages. In most parts of the country the quality of the natural pasture decreases after the occurrence of frost. During the dry season in the tropics forage quality is generally poor, which may limit the rate of fibre digestion (Romney & Gill, 2000). Post harvest grain crop residues, such as wheat straw, oat straw, maize stover and dry standing hay are normally available in large quantities, but are low in nutritive value as they consist mainly of highly lignified stems (Meissner, 1997). Mature forages and cereal crop residues therefore have a common characteristic of being bulky due to the high fibrous and lignin content and are poorly consumed by ruminants (Van Soest, 1994). Physical and chemical treatments have been used for fibrous crop residues, such as straws, to improve their digestibility and contribute to the energy requirement of productive ruminants (Owen & Jayasuriya, 1989). Feed additives (Varga & Kolver, 1997) have also been applied to low quality forages such as straws to improve their intake and rate of digestion by ruminants. Romney & Gill (2000) stated that good quality legumes, such as lucerne hay, and concentrates are supplemented to improve utilization of low quality forages.

Scientists have examined the impact of genetic manipulation of microbial species (Wallace, 1994) and defaunation of protozoa in the rumen microbial population (Van Soest, 1994) to influence the balance of fermentation products. Manipulation of rumen fermentation by increasing the number or cellulolytic activity in the rumen was practiced in increasing degradation of poor quality forages (Gordon *et al*., 1995). However, in the last years the use of fibrolytic enzymes as feed additives has received considerable attention

Lewis *et al*. (1996) and Rode *et al*. (1999) examined the use of exogenous fibrolytic enzymes to improve forage digestion. Improvements in rumen degradability of fibre have been reported (Lewis *et al*., 1996) when exogenous fibrolytic enzymes were added to diets, but others have reported no effect of exogenous enzyme addition on rumen fermentation (Hristov *et al*., 1996). Krause *et al*. (1998) reported a 28% improvement in acid detergent fibre (ADF) digestibility when exogenous enzymes containing xylanase activity were added to a high-concentrate diet. ZoBell *et al*. (2000) reported no effects when the same enzyme product was added to a high-grain barley-based feedlot finishing diet containing 17% forage on a dry matter basis. Although positive, as well as no effects were reported in the literature, results from research on the effects of exogenous fibrolytic enzymes on ruminants diets are variable and not conclusive (Beauchemin *et al*., 2003).

Enzymes are substrate specific, and researchers at Department of Microbiology (Stellenbosch University) decided to cultivate fungal enzymes on a local substrate (wheat straw) in an attempt to develop exogenous fibrolytic enzyme products to enhance digestibility of local forages. Goosen (2004) screened more than 200 fungal extracts obtained from the Department of Microbiology (Stellenbosch University) and the supernatant harvested from one of the strains (ABO374) showed positive results which suggested potential for the improvement of *in vitro* degradation of wheat straw. Cruywagen & Goosen (2004) reported improved growth rates and feed conversion ratios in growing lambs consuming a diet pre-treated with the exogenous enzyme ABO374. However, further research is needed to determine the effects of exogenous enzymes on degradability and fermentation characteristics of mature forages and complete diets containing high or low levels of forages.

The objective of this study was to evaluate the effects of exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) on *in vitro* dry matter (DM) and NDF degradation and gas production (GP) kinetics of mature forages and mixed feed substrates with three levels of concentrate inclusion.

REFERENCES

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 (Suppl. 2), E37-E47.

Cruywagen, C.W. & Goosen, L., 2004. Effect of an exogenous fibrolytic enzyme on growth rate, feed intake and feed conversion ratio in growing lambs. S. Afr. J. Anim. Sci. 34 (Suppl. 2), 71-73.

Gordon, G.L.R., McSweeney, C.S. & Phillips M.W., 1995. An important role for rumen anaerobic fungi in the voluntary intake of poor quality forages by ruminants. In: Rumen ecology research planning. Wallace, R.J. & Lahlou-Kassi, A. (Eds.), Proc. Workshop, Addis Ababa, Ethiopia, 13-18 March, pp. 91-102.

Goosen, L., 2004. The effect of an exogenous fibrolytic enzyme on forage digestibility parameters. M.Sc. (Agric) thesis, University of Stellenbosch, Stellenbosch, South Africa.

Hristov, A.N., Rode, L.M., Beauchemin, K.A & Wuerfel, R.L., 1996. Effect of a commercial enzyme preparation on barley silage *in vitro* and *in sacco* dry matter degradability. Proc. Western Sec. Amer. Soc. Anim. Sci. Rapid city, South Dakota 47, 282-284.

Hungate, R.E., 1966. The rumen and its microbes. Academic press, NY.

Krause, M., Beauchemin, K.A., Rode L.M., Farr, B.I. & Nørgaard, P., 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Anim. Sci. 76, 2912-2920.

Lewis, G.E., Hunt, C.W., Sanchez, W.K., Treacher, R., Pritchard, G.T. & Feng, P., 1996. Effects of direct-fed fibrolytic enzymes on digestive characteristics of a forage based diet fed to beef steers. J. Anim. Sci. 74, 3020-3028.

Meissner, H.H., 1997. Recent research on forage utilization by ruminant livestock in South Africa. Anim. Feed Sci. Technol. 69, 103-119.

Mertens, D.R., 1997. Creating systems for meeting fiber requirements of dairy cows. J. Dairy Sci. 80, 1463-1481.

Owen, E. & Jayasuriya, M.C.N., 1989. Use of crop residues as animal feeds in developing countries. Res. Develop. Agric. 6, 129-138.

Rode, L.M., Yang, W.Z. & Beauchemin, K.A., 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. 82, 2121-2126.

Romney, D.L. & Gill, M., 2000. Intake of forages. In: Forage Evaluation in Ruminant Nutrition. Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M. (Eds.), CABI International, Wallingford, UK, pp. 43-61.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. 2nd edition. Cornell University Press, Ithaca, NY.

Varga, G.A. & Kolver, E.S., 1997. Microbial and animal limitations to fiber digestion and utilization. J. Nutr. 127, 819S-823S (Abstr.).

Wallace R.J., 1994. Ruminal microbiology, biotechnology, and ruminant nutrition: progress and problems. J. Anim. Sci. 72, 2992-3003.

ZoBell, D.R., Weidmeier, R.D., Olson, K.C. & Treacher, R.J., 2000. The effect of an exogenous enzyme treatment on production and carcass characteristics of growing and finishing steers. Anim. Feed Sci. Technol. 87, 279-285.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Forages are the major component of ruminant rations throughout the production systems in the world. They are consumed by ruminants in a variety of situations, ranging from grazing forages to consumption of processed forage as a component of total mixed diets (Romney & Gill, 2000). Forages, such as straws, are a potential feed for ruminant animals, as ruminants are best adapted to the utilization of plant cell walls (Hungate, 1966) for conversion of fibrous feed sources into milk and meat products.

However, the efficiency of utilization of forages for meat and milk production depends on the digestibility of forage cell walls (Beauchemin *et al*., 2004a). Plant cell walls comprise about 40 to 70% of the dry matter in forages, and cell wall digestibility is generally less than 65% (Van Soest, 1994), even with cultivated forages. Beauchemin *et al*. (2001) stated that when conditions in the rumen are suboptimal (low pH 5.4-6.0) for fibre digestion due to high grain diets, plant cell wall digestion in the total tract contributes only 50%, with 35% ruminal digestion and the rest is digested in the hindgut.

As plants age, they become mature and decline in nutritive value as a result of increased lignification (Van Soest, 1994). Mature plants consist mostly of stem material and less leafy parts. Leaves of grasses and herbaceous plants change quality due to the aging process. Van Soest (1994) stated that the extent to which maturity occurs is dependent on the type of forage and growing environmental factors such as temperature, light and water. For example, temperate forages tend to have better digestibility than tropical plants, hence the need to improve digestibility of tropical forages.

The use of exogenous enzymes to improve forage utilization and digestibility was first examined for ruminant performance in the 1960's (Burroughs *et al*., 1960). These authors reported improved weight gains of 7 to 24% and improved feed conversion ratio's of 6 to 21% when forage (lucerne

hay) was treated with an exogenous enzyme cocktail containing cellulolytic enzymes (Agrozyme, supplied by Merk Sharp and Dohme Research Laboratories, and contained both amylolytic and proteolytic enzymes and others). An increased weight gain of 14% compared with the control was also reported by Nelson & Damon (1960) when cattle received a maize-lucerne hay diet treated with four different exogenous preparations. Letherwood *et al*. (1960) reported no improvement in weight gain or feed utilization when a fungal enzyme extract was added to a grain-lucerne hay diet. Enzyme cocktails containing cellulose enzymes have also resulted in a reduction in animal performance when applied to forage based diets fed to ruminants (Clark *et al*, 1961; Perry *et al*., 1966). Hristov *et al*. (1998) revealed that addition of a fibrolytic enzyme resulted in increased concentration of soluble reducing sugars and decreased NDF content. Feng *et al*., (1996) reported an increased *in vitro* dry matter and organic matter digestibility when exogenous enzymes were treated to mature grass forage fed to beef steers. Improvements in digestibility of dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), and acid detergent fiber (ADF) in Awassi lambs fed a concentrate based diet supplemented with fibrolytic enzymes were also reported (Titi & Tabbaa , 2004).

Beauchemin & Rode (1996) noted that most of the early enzyme products for ruminants were poorly characterized and responses were variable. Availability of more active and better defined exogenous enzyme products prompted researchers (Feng *et al*., 1996; Krause *et al*., 1998 and Rode *et al*., 1999) to re-examine the potential use of exogenous enzymes in ruminant diets. Some progress has been made in using existing exogenous enzyme products, such as cellulases, hemicellulases and xylanases for ruminant diets. An understanding of rumen fibre utilization, anaerobic gas production, and the degrading mechanism of exogenous enzymes are necessary to ensure effective and consistent results. This review will discuss the issues highlighted in this introduction. Factors affecting exogenous enzyme action will also be discussed.

2.2 WHAT IS CONSIDERED A FORAGE?

Forage is defined as the edible parts of plants, other than separated grain that can provide feed for grazing animals or that can be harvested for feeding (Forage & Grazing Terminology Committee, 1991). This definition includes the classes of feed such as herbage, hay and silage, browse and straws (Wilkins, 2000). The narrower term ‘forage crop’ is often used to describe crops, commonly

annual or biennial, which are grown to be utilized by grazing or harvested as a whole crop (for example maize and sorghum). Forage consists largely of carbohydrate in the form of fibre, and its digestion is accomplished through the enzymic action of the rumen microbes. A wide range of plant feeds have substantial cell wall content (root crops are an exception) and are suited to utilization by ruminants with their substantial capability of cell wall component digestion by rumen microbes (Wilkins, 2000).

The nutritive value of forages is particularly variable due to variation in plants, soils and weather conditions. Range in energy and protein content of different classes of forages is illustrated in Table 1.1.

Table 1.1 Range in energy (MJ/kg DM) and protein (g/kg DM) content of different classes of forages.

Type of class	Metabolizable energy (MJ/kg DM)	Crude protein (g/kg DM)
Temperate grasses, hays and silages	7.0 - 13.0	60 – 250
Tropical grasses	5 – 11	20 – 200
Cereal straw	5 – 8	20 – 40
Root crops	11 – 14	40 – 130
Kale and rape	9 - 12	140 - 220

Source: Wilkins (2000)

2.3 UTILIZATION OF FIBRE BY RUMINANTS

Fibre is defined as the slowly digestible or indigestible fraction of feeds that occupies space in the gastrointestinal tract of ruminant animals (Mertens, 1997). Knowlton (2003) stated that fibre utilization by high producing dairy cattle comprises about 25 to 35%, which increases the energy available in their rations. Ruminants depend upon microbial fermentation of feed to extract nutrients from ingested plant materials. The relationship is symbiotic as the animal provides a suitable habitat

for microbial growth, and the animal utilizes end products of microbial fermentation (Hungate, 1966).

Ruminants regurgitate and chew feed to reduce particle size (Wilson & Kennedy, 1996). Saliva secreted during rumination helps in stimulating chewing activity and also buffers the ruminal liquor in order to maintain optimal ruminal pH. Jung & Allen (1995) stated that a reduction in the size of cell wall material increases digestibility and nutrient availability in the feed. Rumen microbes enter interior cells of forages through stomata, fractures in the cuticle or through cut or sheared surfaces by adhesion via protein complexes (Varga & Kolver, 1997). Adhesion is followed by successive microbial colonization within the adherent population until active digestive consortia are formed and nutrients are released from the digestion of the substrate (Cheng *et al*., 1991). Microbes, by attaching themselves to fibre particles not only increase their ability to deliver enzymes, but are also able to extend their residence time within the rumen by avoiding passage through the reticulorumen (Omed *et al*., 2000).

Utilization of fibre in mature forages by rumen microbes is slow and incomplete because of high cell wall and lignin content (Van Soest, 1994). Wilson & Kennedy (1996) stated that physical and structural barriers such as waxes and the cuticle of the epidermis limit rumen microbes and enzymes access to tissues of mature forages.

The two major limitations in rumen fibre utilization are the rate and extent of plant cell hydrolysis (Gilbert & Hazelwood, 1991). Demeyer (1981) stated that the total amount of cellulose and hemicellulose degraded in the rumen depends on the degree of lignification which is related to the maturity of the forage. The ruminal pH also contributes to the limitation of fibre digestion in the rumen by reducing fibre digestion through its influence on the growth rates and activity of cellulolytic microbes when conditions are suboptimal (Weimer, 1998). Growth rates of cellulolytic microbes are optimal at a ruminal of pH 6.2 to 6.8 and reduction in that pH reduces fibre digestion (Zinn & Salinas, 1999). The NRC (2001) recommends that the fibre contents of diets be adjusted to ensure the stimulation of chewing activity, salivation and maintenance of an optimal ruminal pH to enable degrading enzymes to interact with the target substrates.

2.3.1 Rumen fermentation products

The major end-products of fibre utilization by rumen microbes are volatile fatty acids (VFA; acetic, propionic, butyric), microbial crude protein (MCP), carbon dioxide (CO_2) and methane (CH_4). The VFA are absorbed through the rumen wall, and serves as the primary source of energy for mucosal tissue and the host animal. According to the NRC (2001), absorbed VFA may supply up to 75 to 80% of the animal's digestible energy requirement, while MCP leaving the rumen may account for 64% of the CP digested and absorbed in the small intestine.

High fibre diets result in high levels of acetate and butyrate, while high cereal diets promote high levels of propionate (Beever & Mould, 2000). Acetate is essential as a precursor in milk fat synthesis in dairy rations (Mertens, 1997); and propionate is the primary precursor for synthesis of glucose by the liver (Knowlton, 2003). Butyrate is used as an energy source and for milk fat synthesis (Van Soest, 1994). Branched VFA (iso-butyrate and iso-leucine) serve as carbon skeletons for MP synthesis (Beever, 1993). Gases produced from VFA production are eructated to the atmosphere. Fibre digestion of mature forages results in low synthesis of MP from the rumen because of their slow and incomplete digestibility. Ceacal fermentation products are lost through fecal excreta (Van Soest, 1994).

2.4 *IN VITRO* ANAEROBIC GAS PRODUCTION

The basic principle of gas production is that the *in vitro* fermentation of feeds incubated with buffered rumen fluid is accompanied by the production of gas (Awati *et al*., 2006). The gas is formed directly by microbial fermentation of the substrate as well as indirectly by release of carbon dioxide caused by production of VFA from the bicarbonate buffer (Van Soest, 1994). However, it is very difficult to separate direct vs. indirect gas production. Based on the fact that both are directly related to the fermentation of a substrate, the gas production measured at each time point can be considered as an index for fermentation activity (Groot *et al*., 1996).

Gas production is the result of fermentation of carbohydrates to VFA (Blümmel & Ørskov, 1993). Gas production from protein fermentation is relatively small as compared to carbohydrate

fermentation (Wolin, 1960). The three reactions giving rise to the main end-products of carbohydrate fermentation in the rumen was summarized by Hungate (1966) as follows:

1 mol Hexose +2 H_2O	→	2 Acetate +2 CO_2 + 4 H_2	(1)
1 mol Hexose +2 H_2	→	2 Propionate + 2 H_2O	(2)
1 mol Hexose	→	1 Butyrate + 2 CO_2 + 2 H_2	(3)

The reaction equations above show that the total amount of gas produced in gas production differs depending on both the amount of substrate fermented and the amount and molar proportions of the VFA end- products formed (Davies *et al*., 2000). Rapidly fermentable carbohydrates yield relatively higher amounts of propionate as compared to acetate, and the reverse takes place when slowly fermentable carbohydrates are incubated (Van Soest, 1994). Getachew *et a*l., (1998) stated that more propionate and lower acetate ratios in the rumen fluid of cows fed a high grain diet were reported by researchers. The gases produced from VFA production and from the bicarbonate buffer in the rumen are eructed to the atmosphere. It is this gas that is being measured *in vitro*.

2.5 DEVELOPMENTS IN ENHANCING FIBRE UTILIZATION AND DIGESTION

There are a number of well-established strategies that have been attempted by Scientist to improve fibre utilization and digestibility through the use of different technologies before plant cell material is consumed by ruminants. Several methods such as physical, chemical (sodium hydroxide and ammonia, and urea supplements) and biological treatments have been used to improve the nutritive value of forages and by-products (Wilkins & Minson, 1970; Sundstol, 1988). Oji *et al.,* (1977) have stated that the final effect for using these treatments is an increase of the digestibility of dry matter (DM) and cell wall, nitrogen content and DM intake. Wylie & Steen (1988) used ammonia in treating forages to solubilize hemicellulose (increasing digestibility of the energy) and lignin, and to improve the amount of available nitrogen for microbes. However these treatments are now less commonly used because of new developments on opportunities to improve forage utilization by ruminants (Varga & Kolver, 1997).

The use of genetic selection for decreased fibre concentration or improved rate or extent of fibre digestion was also practiced (Jung & Allen, 1995). Decreasing fibre concentration may increase DM intake and digestibility of forage.

Krause *et al*. (2003) reviewed the use of genetically modified fibre-degrading enzymes, modification of fibre by the use of plant genetic manipulation and application of free-living lignolytic fungi, and use of exogenous enzymes to improve fibre degradation in the rumen. Agosin *et al*. (1985) reported that *in vitro* digestibility of wheat straw increased from 38 to 68% when treated with strains of lignolytic fungi. In comparison with controls, cattle gained weight (6.8-24%) and converted feed more efficiently (6.0 to 1.2%) when ground maize, oat silage, maize silage or lucern hay was treated with an enzyme cocktail containing amylolytic, proteolytic and cellulolytic enzymes (Krause *et al*., 2003). Beauchemin *et al*. (2004a) have been critical of improving cell wall quality and digestibility exclusively through forage breeding programs and management. However, Jafari *et al*. (2005) found that ionophores, direct fed microbials and exogenous degrading enzymes improved digestibility of poor quality (fibrous) forages in ruminant livestock. Tricarico & Dawson (1999) reported that the addition of xylanase and cellulase enzyme preparations directly into feed improved the *in vitro* rumen digestion. Zinn & Salinas (1999) reported that a rumen-stable fibrolytic enzyme supplement increased the rumen digestion of NDF and Feed N by 23 and 5%, respectively. They also reported an improvement in dry matter intake and average daily gain in steers supplemented with this additive. Despite these attempts and improvements, forage quality continues to limit fibre digestibility and intake of nutrients by ruminants (Beauchemin *et al*., 2004a).

Research in applying exogenous fibre degrading enzymes to enhance rumen digestive activity of forages (Jafari *et al*., 2005) and assessing the effects and efficiency of exogenous enzymes *in vitro* (Eun *et al*., 2007), still continues. Eun *et al*. (2007) reported improvements in NDF and ADF degradation by 9.6% and 25.6% respectively, when lucerne hay was treated with exogenous enzymes containing endoglucanase and xylanase activities. Based on the findings by the researchers, exogenous enzymes may have potential in improving fibre utilization by ruminants; hence its evaluation still continues.

2.6 DEGRADING MECHANISMS BY EXOGENOUS ENZYMES

2.6.1 Pre-treatment effects

There is evidence that pre-treatment of plant fibre with exogenous enzymes allows the enzyme to bind to the target substrate (Beauchemin *et al*., 2003), thereby increasing resistance to proteolysis in the rumen. Hristov *et al*. (1996) stated that pre-treatment effects to feed cause the release of soluble carbohydrates to the rumen microbes. The release of sugars from feeds due to exogenous enzymes is partially the result of NDF and ADF solubilization (Hristov *et al*., 2000). Nsereko *et al*. (2000) reported structural changes caused by treatment with exogenous enzymes to feed resulting in feed being more available for degradation in the rumen. However, this evidence does not account for improved dietary fibre digestion when exogenous enzymes are applied to the concentrate portion of the diet (Yang *et al*., 2000).

Feng *et al*. (1992) reported that pre-treatment of dry grass with exogenous enzymes improved *in vitro* fibre digestion. Improvements in VFA production and NDF digestion were reported by Lewis *et al*. (1996) when exogenous enzymes were sprayed onto a grass hay-barley diet prior to feeding. Cruywagen & Goosen (2004) reported improved weight gain (6.75 and 7.13kg) and feed conversion ratios (0.15 and 0.16kg gain/kg DMI) when wheat straw was pre-treated with exogenous enzyme ABO374 for 18 h before feeding to growing lambs at high and medium levels of enzyme application, respectively.

Over and under-treatment of feeds with exogenous enzymes may result in the blocking of binding sites for enzymes or may prevent substrate colonization by rumen microbes, and thus lead to a reduction in the activities of the enzymes (Beauchemin *et al*., 2003).

2.6.2 Synergy between exogenous fibrolytic enzymes and rumen microbes

Exogenous enzymes are extracellular fermentation products of fungal origin (Pendleton, 2000), developed to degrade plant structural cell wall fractions such as cellulose, hemicellulose and lignin into small fractions. Beauchemin *et al*. (1999) and Titi & Tabbaa (2004) reported that exogenous enzymes provide a slow sugar release mechanism by breaking down the fibrous complex in plant

structural carbohydrates, releasing nutrients for rumen microbes. However, the degree of sugar release is dependent on feed and enzyme type (Krause *et al.*, 2003). Beauchemin *et al*. (2003) stated that exogenous enzymes activities form a unique stable feed enzyme complex which prevents them from proteolysis in the rumen. Rumen microbes have also evolved to digest plant fiber, and they possess a vast array of enzymes that are able to hydrolyze plant structural polysaccharides (Forsberg *et al*., 1997). Therefore, for exogenous fibrolytic enzymes to positively enhance feed digestion in the rumen, they would have to contain enzymic activity that is limiting the rate of the hydrolysis reaction (Morgavi *et al.,* 2000).

Synergy between exogenous enzymes and rumen microbe enzymes can be defined as the enhanced effect of these two entities acting cooperatively (Morgavi *et al*., 2000). The net effect is the increase in enzymatic activity that exceeds the additive effects of each of the individual components. McAllister *et al*. (2000) stated that synergism may be observed when rumen microbes are unable to degrade target substrates or when conditions in the rumen are below a pH of 6.2. Exogenous enzymes differ from rumen microbe enzymes by having lower optimal pH (Morgavi *et al*., 2000). Beauchemin *et al*., (2004a) stated that when enzymes from *Trichoderma longibrachiatum* were combined with ruminal enzymes extracted from cattle fed high fibre or high concentrate diets, hydrolysis of soluble cellulose and xylan increased by 35 and 100%, respectively. The authors also stated that hydrolysis of corn silage also increased by 40% when the same enzymes from *Trichoderma longibrachiatum* were combined with ruminal enzymes. Colombatto *et al*. (2003) reported higher improvements of 43 and 25% in NDF degradability when exogenous enzymes were added at high and low pH of 6.0 to 6.6 and a pH of 5.4 to 6.0, respectively. Thus, synergism may be observed between rumen microbes and exogenous fibrolytic enzymes, because exogenous fibrolytic enzymes may help improve fibre digestion when conditions are suboptimal, but they are not expected to fully overcome the limits to digestion imposed by low ruminal pH.

Morgavi *et al*. (2000) reported a synergy between exogenous fibrolytic enzymes and rumen enzymes when enzymes from *Trichoderma longibrachiatum* were combined with rumen enzymes receiving high fibre or high concentrate diets. The feed hydrolysis increased by up to 35, 40 and 100% in the case of soluble cellulose, corn silage and xylan, respectively. Wang *et al*. (2001) reported increased hydrolytic capacity of the rumen when supplementing feed with exogenous enzymes, which improved microbial attachment of rumen microbes to feed and increased enzyme activity; thus enhancing digestibility of the diet. Improved hydrolytic capacity of the rumen were

also reported by Beauchemin *et al*. (2003) when exogenous enzymes were added to the concentrate portion of the diet, which improved digestibility of concentrates and fibre components of the diet.

Improvement in rumen digestion of fibre reported with the use of exogenous fibrolytic enzymes in ruminant diets partially indicates the cooperative effect between exogenous enzymes and rumen microbes. However, the improvement explains the positive responses observed when exogenous enzymes are supplemented to ruminant diets.

2.7 FACTORS AFFECTING EXOGENOUS ENZYMES ACTION AND ACTIVITY

Rumen conditions can cause a reduction of exogenous enzymes activities such that responses in fibre digestion and production are not obtained following enzyme application (Vicini *et al*., 2003). Enzyme activities are dependant on several factors such as ruminal pH, temperature, concentration of the enzyme, target substrate (Adesogan, 2005), as well as culture conditions employed (Gashe, 1992). To properly assess its activities, Colombatto & Beauchemin (2003) reported that the enzyme activities should be tested under the conditions it will be used. Enzyme activity is assessed by measuring over time either the disappearance of a defined substrate or generation of a product from a biochemical reaction catalysed by enzyme (McAllister *et a*l., 2001). Activities of enzymes are expressed as the amount of product produced per unit time. Fibre degrading enzyme activity are determined by measuring the rate of release of reducing sugars from substrates, with enzyme units expressed as the quantity of reducing sugars released per time/unit enzyme (µmol glucose/min/m L) (Beauchemin *et al*., 2004b). These authors have also stated that in enzyme assays, substrate should be in excess to prevent the reaction from reaching stability and therefore loosing linearity of reaction rate and time.

Eun & Beauchemin (2007) stated that a relationship between the activity of the exogenous enzyme and the substrate is important for degradation of fibre to occur. The lack of response from substrate degradation may be associated with exogenous enzymes not being stable or low in activity. Vicini *et al*. (2003) reported a lack of response to enzyme treatment due to high ruminal pH (6.8) and low temperature (32°C) compared to the optima for the exogenous enzyme activities in their preparations. Hence, variation in enzymic activities may be expected when enzyme preparations are assessed under optimal conditions versus rumen conditions.

Insufficient or in excess amounts of enzyme products may be ineffective if ideally formulated. Beauchemmin *et al*. (1995) added incremental amounts of an enzyme product (Xylanase B, Biovance, Technol., Omaha, NE, combined with Spezyme CP, Genencor, Rochesteer, NY) to lucern hay, timothy hay or whole crop barley silage. The authors reported effect of enzyme differed among forages due to enzyme-feed specificity, and the optimum amount of enzyme differed for the forages. For lucern hay, the average daily gain of growing cattle increased by 24 to 30% with lower application rates of added enzyme at (0.25 to 1 mL/kg DM) as a result of increased intake of digestible DM, but higher application rates (2 and 4 mL/kg DM) were not effective. (Beauchemin *et al*., 2004b) have stated that larger amount of enzyme supplementation can be less effective than smaller amounts and that the optimum amount of enzyme supplementation depends on the diet. The lack of response to low concentrations of enzymes supplementation indicates an insufficient supply of enzyme activity. At times, it is possible that exogenous enzymes compete with the rumen population for cellulose binding sites available to feeds (Beauchemin *et al*., 2004b)

2.8 *IN VITRO* TECHNIQUES FOR ASSESSING EFFECTS OF EXOGENOUS ENZYMES

The two-stage technique (Tilley & Terry, 1963) is used in many forage evaluation laboratories and involves two steps in which forages are subjected first to fermentation *in vitro* with rumen fluid followed by a digestion with pepsin in a weak acid for predicting in vivo digestibility (De Boever *et al*., 1988). The technique, however, has a disadvantage in that it uses donor animals, and does not provide information on the kinetics of forage digestion (Theodorou *et al*., 1994) but only an end point measurement. To enhance post rumen digestibility, the method was modified by Goering & Van Soest (1970) by treating the residue with ND solution to estimate true DM digestibility (Beever & Mould, 2000). Cellulose base techniques (enzymic method) have been used with success to estimate forage digestibility. As with the Tilley & Terry method, evaluations are also generally used as end point digestibility procedures, and therefore do not provide information on kinetics of forage digestion. The main advantage of the enzymic method is that it does not require animal donors (Theodorou *et al*., 1994), and a disadvantage is the problem with variability in enzyme activity.

A filter bag technique developed by ANKOM Technology (Fairport, NY) was introduced to simplify the evaluation of *in vitro* digestibility. The method involves digesting forage samples weighed into dacron bags, suspended in a mixture of buffered medium solution and rumen fluid

within rotating digestive jars in an insulated incubator (DAISY^{II} incubator). The incubator is designed to utilize dried and ground material, which represent the chemical properties of feeds (Beauchemin *et al*., 1999). The technique gives relatively accurate predictions on in vitro apparent and true digestibility (Wilman & Adesogan, 2000). The filter bag technique has a potential to estimate the degradation rates of feeds. It reduces labor input associated with *in vitro* digestibility estimation because it prevents the need for filtration. Holden (1999) reported that the DAISY^{II} method provided good correlations with results obtained from the two-stage method in estimating DM digestibility of more than one type of feed. Different forages, grains and mixed feeds can be analyzed together in a single digestion jar. The DAISY^{II} method represents a faster, convenient way to determine *in vitro* digestibility of feeds.

In vitro gas production techniques (IVGPT) generate kinetic data rather than measuring the disappearance of digested feeds. The technique measures the appearance of the fermentation gases particularly CO_2 and CH_4 (Getachew *et al*., 2004). Gas production methods have been used in determining fermentation kinetics of rates and extent of digestion, VFA production and microbial protein production (Pell *et al*., 1998) of feeds. Wilkins (1974) described a GP technique to measure fermentation kinetics *in vitro*. A sealed jar was used and gas produced was determined using a pressure transducer to measure the accumulation of pressure in the jar headspace. This standard of measuring pressure with a sensor or transducer has been widely used as method of determining fermentation kinetics.

The simplest pressure measurement technique requires manual measurement of headspace pressure, as described by Theodorou *et al*. (1994). Pell & Schofield (1993) and Davies *et al*. (2000) described the semi- and full automation of headspace pressure recording. Gas production describes the kinetics of microbial activity in response to a given substrate with a given microbial population, thereby giving a practical imitation of what occurs in the rumen. The method is a useful tool for the evaluation of ruminant feedstuffs. One limitation to the use of the technique for forage evaluation is the lack of uniformity in methodology. Factors such as anaerobisis, temperature and pH, and adequate buffering may affect the gas production of feeds (Getachew *et al*., 1997).

Advantages of *in vitro* methods are that they are less time-consuming and less costly. Feed samples or feed components can be studied in isolation, and smaller quantities of feed are required. A limitation of the general use of *in vitro* methods is that they require donor animals.

2.10 IMPROVING THE USE OF EXOGENOUS ENZYME EXTRACTS ON RUMINANT DIETS

The principal rationale for the use of enzymes is to improve the nutritive value of feedstuffs by increasing the efficiency of feed utilization in ruminants and reduce waste production (Beauchemin *et al*., 2004b). Not all exogenous enzymes are effective at digesting complex substrates such as forages and concentrates. These substrates are structurally complex materials, and lack of information of the factors that limit the rate and extent of feed digestion delays the use of exogenous enzyme preparations designed to overcome constraints of feed digestion (McAllister *et al*., 2001).

With some feeds, specific targets can be identified. Maize consists of a protein matrix surrounding the starch granules, which determines the extent and rate of starch digestion in the grain (McAllister *et al*., 1993). Exogenous enzymes designed to improve the utilization of maize may contain proteases capable of digesting the protein matrix and exposing starch granules to digestion by rumen enzymes. Bae *et al*. (1997) stated that the primary limiting factors to microbial digestion in straws are silica, lignin and cutin.

Opportunities to improve the utilization of these materials for ruminants exist. McAllister *et al*. (2001) stated that recent developments in biotechnology now make it possible to prepare specific enzyme cocktails for different feed types. Anaerobic fungi in the rumen are able to penetrate plant tissue as a result of their filamentous growth and degrade lignin in plant tissue (McSweetney *et al*., 1994). Agosin *et al*. (1985) reported that *in vitro* digestibility of wheat straw improved from 38% to 68% when treated with lignolytic fungal strains. Exogenous enzymes such as lignolytic enzymes with the capacity to attack the structural barriers in materials involving the lignin-carbohydrate bonds could be isolated and examined for their specific enzymatic and attachment capabilities (Varga & Kolver, 1994; Krause *et al*. 2003).

Exogenous cell wall degrading enzymes is an emerging technology that shows potential in terms of improving the utilization of forages by ruminants. Improving the use of these enzymes may enhance in forage fibre digestion the availability of energy to ruminants (Feng *et al*., 1996). (McAllister *et al*., 2001) stated that because most of these exogenous enzymes are often overlooked or poorly defined before use it is uncertain which, if any, enzyme activity limits the rate and extent of degradation in the rumen. Further research is required to clarify the important factors to consider

reducing the variability associated with using exogenous enzymes in ruminant diets. As suggested by Wallace *et al*. (2001), an identification of the enzymatic activity causing a positive response in fibre digestion and rumen fermentation might make possible to develop more effective fibrolytic enzyme products.

2.11 CONCLUSION

The use of exogenous fibrolytic enzymes as an emerging technology has potential for improving forage utilization, fibre digestion and production by ruminants. It is evident from many studies that exogenous enzymes applications are effective in enhancing fibre digestion and animal production provided that the proper environment, suitable temperature, ruminal pH and target substrate are maintained, and that enzymes activities are well defined. Effects of exogenous enzyme application on ruminant feeds seem to vary with the physical and chemical composition of the targeted substrate.

Synergism between the rumen microorganisms and exogenous enzymes has been defined as the release of reducing sugars by exogenous enzymes increasing the activities of fibre digestion in the rumen under low ruminal pH conditions. From the responses reported on synergism between exogenous enzymes and rumen microbes, the effect of exogenous enzymes to enhance fibre digestion seems to be influenced by rumen pH and enzyme activities contained by the enzyme product. Attempts to improve rumen fibre digestion are hampered by the lack of understanding of structural cell wall complexes. It is uncertain whether is the structural cell wall complexes or the enzymes activities applied that are the major limitation in fibre digestion.

Application of exogenous enzymes is based largely on the availability of enzymes to enhance rate and extent of fibre digestion, but information on exogenous enzymes requirements in the rumen is limited. Information regarding the production and preparations of exogenous enzymes in ruminant feeds is also limited. However, research on improving the utilization of mature forage based diets and crop residues for ruminants with exogenous fibrolytic enzyme preparations is in progress. Hopefully, the use of exogenous fibrolytic enzymes to maximize the use of crop residues as ruminant feeds, rate and forage digestion and animal production will play an important role in the future.

The preparation of various exogenous enzymes involves dilutions and proper pH and temperature to maintain its activities. Information regarding the choice of exogenous enzymes, enzyme composition, application method and level, stability, target substrate and storage is needed for better defined effects of exogenous enzymes and to be considered for use in conditions at farm level.

2.11 REFERENCES

Adesogan, A.T., 2005. Improving forage quality and animal performance with fibrolytic enzymes. Florida Ruminant Nutrition Symposium. Department of Animal Sciences, Institute of food and Agricultural Sciences, University of Florida, Gainesville, FL 32611, pp. 91-100.

Agosin, E. & Odier, E., 1985. Solid-state fermentation, lignin degradation and resulting digestibility of wheat straw fermented by selected white-rot fungi. Appl. Microbiol. Biotechnol. 21, 297-403.

Awati, A., Williams, B.A., Bosch, M.W., Li, Y.C. & Verstegen, W.A. 2006. Use of the *in vitro* cumulative gas production technique for pigs: An examination of alterations in fermentation products and substrate losses at various time points. J. Anim. Sci. 84, 1110-1118.

Bae, H.D., McAllister, T.A., Kokko. E.G., Leggett, F.L., Yanke, L.J., Jakober, K.D., Ha, J.K., Shin, H.T & Cheng, K.J., 1997. Effect of silica on the colonization of rice straw by rumen bacteria. Anim. Feed Sci. Technol. 65, 165-181.

Beauchemin, K.A., Rode, L.M. & Sewalt, V.J.H., 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. 75, 641-644.

Beauchemin, K.A. & Rode, L.M., 1996. The potential use of feed enzymes for ruminants. In: Proc. Cornell. Nutr. Conf. Feed. Manuf. Rochester. NY. Cornell University, Ithaca, NY, pp. 131-141.

Beauchemin, K.A., Rode, L.M. & Karren, D., 1999. Use of feed enzymes in feedlot finishing diets. Can. J. Anim. Sci. 79, 243-246.

Beauchemin, K.A., Yang, W.Z & Rode, L.M., 2001. Effects of barley grain processing on the site and extent of digestion of beef feedlot finishing diets. J. Anim. Sci. 79, 1925-1936.

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 (Suppl. 2), E37–E47.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z. & Rode, L.M., 2004a. Mode of action of exogenous cell wall degrading enzymes for ruminants. Can. J. Anim. Sci. 84, 13-22.

Beauchemin, K.A., Colombatto, D. & Morgavi, D.P., 2004b. A rationale for the development of feed enzyme products for ruminants. Can. J. Anim. Sci. 84, 23-36.

Beever, D.E. & Mould, F.L., 2000. Forage evaluation for efficient ruminant livestock production. In: Forage Evaluation in Ruminant Nutrition. Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M. (Eds.), CABI International, Wallingford, UK, pp. 15-42.

Beever, D.E., 1993. Rumen function. In: Quantitative aspects of ruminant digestion and metabolism. Forbes, J.M. & France, J. (Eds.), CABI International, Wallingford, UK, pp. 187-215.

Blümmel, M. & Ørskov, E.R., 1993. Comparison of *in vitro* gas production and nylon bag degradability of roughages in predicting feed intake in cattle. Anim. Feed. Sci. Technol. 40, 109-119.

Burroughs, W., Woods, W., Ewing, S.A., Greig, T. & Theurer, B., 1960. Enzyme additions to fattening cattle rations. J. Anim. Sci. 19, 458-464.

Clark, J.D., Dyer, I.A. & Templeton, J.A., 1961. Some nutritional and physiological effects of enzymes for fattening cattle. J. Anim. Sci. 20, 928-938.

Colombatto, D., & Beauchemin, K.A., 2003. A proposed methodology to standardize the determination of enzymic activities present in enzyme additives used in ruminant diets. Can. J. Anim. Sci. 83, 559-568.

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

Cruywagen, C.W. & Goosen, L., 2004. Effect of an exogenous fibrolytic enzyme on growth rate, feed intake and feed conversion ratio in growing lambs. S. Afr. J. Anim. Sci. 34 (Suppl. 2), 71-73.

Davies, Z.S., Mason, D., Brooks, A.E., Griffith, G.W., Merry, R.J. & Theodorou, M.K., 2000. An automated system for measuring gas production from forages inoculated with rumen fluid and its use in determining the effect of enzymes on grass silage. Anim. Feed Sci. Technol. 83, 205-221.

De Boever, J.L., Cottyn, B.G., Buysse, F.X. & Vanacker, J.M., 1988. The use of a cellulase technique to predict digestibility, metabolizable and net energy of forages. Anim. Feed Sci. Technol. 19, 247-260.

Demeyer, D.I., 1981. Rumen microbes and digestion of plant cell walls. Agric. Environ. 6, 295-337.

Eun, J.S., Beauchemin, K.A. & Schulze, H., 2007. Use of an *in vitro* fermentation bioassay to evaluate improvement in degradation of lucerne hay due to exogenous feed enzymes. Anim. Feed Sci. Technol. 135 (3-4), 315-328.

Eun, J.S. & Beauchemin, K.A., 2007. Assessment of the efficacy of varying experimental exogenous fibrolytic enzymes using *in vitro* fermentation characteristics. . Anim. Feed Sci. Technol. 132 (3-4), 298-315.

Feng, P., Hunt, C. W., Julien, W. E., Dickinson, K. & Moen, T., 1992. Effect of enzyme addition on *in situ* and *in vitro* degradation of mature cool-season grass forage. J. Anim. Sci. 70(Suppl. 1), 309 (Abstr).

Feng, P., Hunt, C.W., Pritchard, G.T. & Julien, W.E., 1996. Effect of enzyme preparations on in situ and *in vitro* degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci. 74, 1349-1357.

Forage & Grazing Terminology Committee, 1991. Vegetation terms. In: Terminology for Grazing Lands and Grazing Animals. Pocahontas Press, Inc., Blacksburg, VA.

Forsberg, C.W., Cheng, K.J & White B. A. 1997. Polysaccharide degradation in the rumen and large intestine: In Gastrointestinal Microbiology, Gastrointestinal Ecosystems and Fermentations. Mackie, R. I. & White, B. A., (Eds.), Chapman & Hall, New York, NY. pp. 1, 319-379.

Gashe, B.A., 1992. Cellulase production and activity by *Trichoderma sp*. A001. J. Appl. Biotechnol. 73, 79-82.

Getachew, G., Makkar, H.P.S & Becker, K., 1997. The *in vitro* gas coupled with ammonia measurement for evaluation of nitrogen degradability in low quality roughages using incubation medium of different measuring capacity. J. Sci. Food Agric (in press).

Getachew, G., Blümmel, M., Mahhar, H.P.S & Becker, K., 1998. *In vitro* gas measuring techniques for assessment of nutritional quality of feeds. Anim. Feed Sci. Technol. 72, 261-281, (review).

Getachew, G., DePeters, E.J. & Robinson, P.H., 2004. *In vitro* gas production provides effective method for assessing ruminant feeds. California Agriculture, 58(1), 54-58.

Gilbert, H.J. & Hazlewood, G.P, 1991. Genetic modification of fibre digestion. Proc. Nutr. Soc. 50, 173-136.

Goering, H.K. & Van Soest, P.J., 1970. Forage fiber analyses (Apparatus, reagents, procedures and some applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC. USA.

Groot, J.C.J., Cone, J.W., Williams, B.A., Debersaques, F.M.A. & Lantinga, E.A., 1996. Multiphasic analysis of gas production kinetics for *in vitro* fermentation of ruminant feeds. Anim. Feed. Sci. Technol. 64, 77-89.

Holden, L.A., 1999. Comparison of methods of *in vitro* dry matter digestibility for ten feeds. J. Dairy Sci. 82, 1791-1794.

Hristov, A.N., Rode, L.M., Beauchemin, K.A & Wuerfel, R.L., 1996. Effect of a commercial enzyme preparation on barley silage *in vitro* and *in sacco* dry matter degradability. Proc. Western Sec. Amer. Soc. Anim. Sci. Rapid city, South Dakota 47, 282-284.

Hristov, A.N., McAllister, T.A. & Cheng, K.J., 1998. Stability of exogenous polysaccharide-degrading enzymes in the rumen. Anim. Feed. Sci. Technol. 76,161-168.

Hristov, A.N., McAllister, T.A., & Cheng, K.J., 2000. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: Effects on nutrient digestion in cattle fed a barley grain diet. J. Anim. Sci. 78, 477-487.

Hungate, R.E., 1966. The rumen and its microbes. Academic press, NY.

Jafari, A., Edriss, M.A., Alikhani, M. & Emtiazi, G., 2005. Effects of treated wheat straw with exogenous fibre-degrading enzymes on wool characteristics of ewe lambs. Pak. J. Nutr. 4(5), 321-326.

Jung, H.G. & Allan, M.S., 1995. Characteristics of plant cell walls affecting intake and digestibility of forages by ruminants. J. Anim Sci. 73, 2774-2790.

Knowlton, K.F., 2003. World of dairy nutrition. Holstein foundation, Department of Animal Science, Virginia Tech. pp. 20-21.

Krause, M., Beauchemin, K.A., Rode, L.M., Farr, B.I. & Nørgaard, P., 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Anim. Sci. 76, 2912-2920.

Krause, D.O., Denman, S.E., Mackie, R.I., Morrison, M., Rae, A.L., Attwood, G.T. & McSweeney, C.S., 2003. Opportunities to improve fibre degradation in the rumen: microbiology, ecology, and genomics. FEMS Microbiol. Rev. 27, 663-693.

Letherwood, J.M., Mochrie, R.D. & Thomas, W.E., 1960. Some effects of a supplementary cellulase preparation of feed utilization by ruminants. J. Dairy Sci. 43, 1460-1464.

Lewis, G.E., Hunt, C.W., Sanchez, W.K., Treacher, R., Pritchard, G.T. & Feng, P., 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J. Anim. Sci. 74, 3020-3028.

McAllister, T.A., Phillippe, R.C., Rode, L.M. & Cheng, K.J., 1993. Effect of the protein matrix on the digestion of cereal grains by rumen microorganisms. J. Anim. Sci. 71, 205-212.

McAllister, T.A., Hristov, K.A., Beauchemin, K.A., Rode, L.M. & Cheng, K.J., 2001. Enzymes in ruminants diets In: Enzymes in farm animal nutrition. Bedford, M.R. & Partridge, G.G. (Eds.), CAB international, Wallingford., UK, pp. 273-298.

McSweetney, C.S., Dulieu, A., Katayama, Y. & Lowry, J.B., 1994. Solubilisation of lignin by the ruminal anaerobic fungus Neocallimastix patriaciarum. Appl. Environ. Microbiol. 60, 2985-2989.

Mertens, D.R., 1997. Creating systems for meeting fiber requirements of dairy cows. J. Dairy Sci. 80, 1463-1481.

Morgavi, D.P., Beauchemin, K.A., Nsereko, V.L., Rode, L.M., Iwaasa, A.D., Yang, W.Z., McAllister, T.A. & Wang, Y., 2000. Synergy between rumen fibrolytic enzymes and enzymes from *Trichoderma Longibrachiatum*. J. Dairy Sci. 83, 1310-1321.

Nelson, F. & Damon, V., 1960. Comparison of different supplemental enzymes with and without diethylstilbestrol for fattening steers. J. Anim. Sci. 19, 1279-1289.

NRC., 2001. Nutrient requirement of dairy cattle. 7th revised. Ed. Natl. Acad. Press. Washington DC, p. 16.

Nsereko, V.L., Morgavi, D.P., Beauchemin, K.A. & McAllister, T.A., 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of lucerne hay fibre by mixed rumen microorganisms *in vitro*. Anim. Feed Sci. Technol. 88,153-170.

Oji, U.I., Mowat, D.N. &Winch, J.E. ,1977. Alkali treatment of corn stover to increase nutritive value. J. Anim. Sci., 44, 79-802.

Omed, H.D., Lovett, D.K. & Axford, R.F.E., 2000. Faeces as a source of microbial enzymes for estimating digestibility. In: Forage evaluation in ruminant nutrition. Givens, D. I., Owen, E., Axford, R. F. E. & Omed, H. M. (Eds), CABI International Wallingford, UK, pp. 135-154.

Pendleton, B. 2000. The regulatory environment. In: Direct-Fed Microbial, Enzyme and Forage Additive Compendium. Muirhea, S. (Ed). The Miller Publishing Co., Minnetonka, MN, p. 49.

Pell, A.N. & Schofield, P., 1993. Computerized monitoring of gas production to measure forage digestion *in vitro*. J. Dairy Sci. 76, 1063-1073.

Pell, A.N., Pitt, R.E., Doane, P.H. & Schofield, P., 1998. The development, use and application of the gas production technique at Cornell University, USA. In: *In vitro* techniques for measuring nutrient supply to ruminants. Deaville, E.R., Owen E., Adesogan, A.T., Rymer, C., Huntington, J.A. & Lawrence, T.L.J. (Eds.), BSAS, Edinburgh, UK, BSAS Occ. Publ. 22, 45-54.

Perry, T.W., Purkhiser, E.D. & Beeson, W.M., 1966. Effects of supplemental enzymes on nitrogen balance, digestibility, or energy and nutrients, and on growth and feed efficiency of cattle. J. Anim. Sci. 25, 760-764.

Rode, L.M., Yang, W.Z. & Beauchemin, K.A., 1999. Fibrolytic enzyme supplements for dairy cows in early lactation. J. Dairy Sci. 82, 2121-2126.

Romney, D.L. & Gill, M., 2000. Intake of forages. In: Forage Evaluation in Ruminant Nutrition. Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M. (Eds.), CABI International, Wallingford, UK, pp. 43-61.

Sundstol, F., 1988. Improvement for low quality forages and roughage. In: World animal science. B. Disciplinary approach feed science, pp. 257-273.

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. Technol. 48, 185-197.

Tilley, J.M.A. & Terry, R.A., 1963. A two-stage technique for the digestion of forage crops. J. Br. Grassl. Sci. 18, 104-111.

Titi, H.H. & Tabbaa, M.J., 2004. Efficacy of exogenous cellulose on digestibility in lambs and growth of dairy calves. Livestock Prod. Sci. 87, 207-214.

Tricario, J.M. & Dawson, K.A., 1999. Effects of defined xylanase and cellulase enzyme preparations on digestive processes of rumen microbial cultures. J. Dairy Sci. 77(Suppl. 1), 252 (Abstr).

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. 2nd edition. Cornell University Press, Ithaca, NY.

Varga, G.A. & Kolver, E.S., 1997. Microbial and animal limitations to fiber digestion and utilization. J. Nutr. 127, 819S-823S (Abstr.).

Vicini, J.L., Bateman, H.G., Bhat, M.K., Clark, J.H., Erdman, R.A., Phipps, R.H., Van Amburgh, M.E., Hartnell, G.F., Hintz, R.L. & Hard, D.L., 2003. Effect of feeding supplemental

fibrolytic enzymes or soluble sugars with malic acid on milk production. J. Dairy Sci. 86, 576-585.

Wallace, R.J., Wallace, S.J.A.., McKain, N., Nsereko, V.L. & Hartnell, G.F., 2001. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed ruminal microorganisms *in vitro*. J. Anim. Sci. 79, 1905-1916.

Wang, Y., McAllister, T.A., Rode, L.M., Beauchemin, K.A., Morgavi, D.P., Nsereko, V.L., Iwaasa, A.D. & Yang, W., 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the rumen simulation technique (Rustec). Brit. J. Nutr. 85, 325-332.

Weimer, P.J., 1998. Manipulating rumen fermentation: A microbial ecological perspective. J. Anim. Sci. 76, 3114-3122.

Wilkins, R. J. & Minson, D.J., 1970. The effects of grinding, supplementation and incubation period on cellulose digestibility *in vitro* and its relationship with cellulose and organic matter digestibility *in vivo*. J. Agric. Sci. 74,445-451.

Wilkins, R.J., 1974. Pressure transducer method for measuring gas production by microorganisms, Appl. Microbiol. 27, 135-140.

Wilkins, R.J., 2000. Forages and their role in animal systems. In: Forage evaluation in ruminant nutrition. Givens, D.I., Owen, E., Axford, R.F.E. & Omed, H.M. (Eds.), CABI International Wallingford, UK, pp. 1-14.

Wilman, D. & Adesogan, A. 2000. A comparison of filter bag methods with conventional tube methods of determining the *in vitro* digestibility of forages. Anim. Feed Sci. Technol. 84, 33-47.

Wilson, J.R. & Kennedy, P.M., 1996. Plant and animal constraint to voluntary feed intake associated with fibre characteristics and particle breakdown and passage in ruminants. Austr. J. Agric. Res. 47, 199-225.

Wolin, M.J., 1960. A theoretical rumen fermentation balance. J. Dairy Sci. 43, 1452-1459.

.Wylie, A. R.G & Steen, R.W.J., 1988. Effect of anhydrous ammonia treatment on the chemical composition and nutritive value of grass hay and on intake and performance in beef steers Grass & Forage Sci.. 43 (1), 79.

Yang, W.Z., Beauchemin, K.A. & Rode, L.M., 2000. A comparison of methods of adding fibrolytic enzymes to lactating dairy diets. J. Dairy Sci. 11, 1-9.

Zinn, R.A., & Salinas, J., 1999. Influence of Fibrozyme on digestive function and growth performance of feedlot steers fed a 78% concentrate growing diet. Proc. Alltech 15th Annual Symposium. Biotechnology in the Feed Industry. Nottingham University Press, pp. 313-319.

CHAPTER 3

The effect of exogenous fibrolytic enzyme application on *in vitro* degradability and gas production characteristics of mature forage substrates

Abstract

In vitro studies were conducted to evaluate the effect of exogenous fibrolytic enzymes on *in vitro* dry matter (DM) and neutral detergent fibre (NDF) degradation, and gas production (GP) of mature forages. Two enzyme cocktails, SU-ABO374 and CSIR-ABO374, were cultivated in fungi on a local substrate (wheat straw) by the Microbiology Department (Stellenbosch University) and CSIR®(Council for Scientific Industrial Research), respectively. These, as well as a control treatment (no enzymes), were applied to three mature forages, lucerne hay (LH), oat hay (OH) and wheat straw (WS). Rumen fluid was collected from two ruminally cannulated Döhne Merino whethers fed a 50:50 mix of lucerne hay and wheat straw supplemented with a concentrate (500g/day). For the DM and NDF degradability trials, 500 mg forage samples were weighed into 50 x 50 mm dacron bags which were incubated anaerobically at 39ºC in 1.4L of a rumen liquid inoculated buffered medium in 2L fermentation jars. Bags from all treatments were removed after 2, 4, 8, 12, 24, 48, 72 and 96 h of incubation. For the gas production determinations, 500 mg forage substrate samples were weighed into 120 ml glass vials which were incubated for 96 h in 40 ml inoculated medium to which 0.5 ml of the respective enzyme solutions were added. Gas pressure was recorded manually with a digital pressure gauge after 2, 4, 8, 12, 24, 48, 72 and 96 h and pressure was converted to volume with a predetermined regression. The 96 h substrate residues were washed, dried, weighed and analyzed for NDF and OM. Enzyme treatments did not affect DM and NDF degradation or GP of forages. The different forages differed in terms of NDF degradation and gas production, but the enzyme treatments had no effect.

(*Key words*: Fibrolytic enzymes, forages, NDF degradability, gas production)

3.1 INTRODUCTION

Utilization of forages by ruminant animals for meat, milk and wool production depends on the availability and quality of the grasses and legumes. Degradation of forage by rumen microbes is limited by the physical characteristics and chemical composition of the forage (Mertens, 1997). Improving the degradation of forages with a high fibre content and low digestibility is essential to increase their energy intake by ruminants (Giraldo *et al*., 2007). Van Soest (1994) stated that physical and chemical processing enhanced availability of cell wall material for degradation by rumen microbes.

Studies were done with exogenous fibrolytic enzymes (McAllister *et al*., 2001; Phipps *et al*., 2002) to increase the degradability of feed in the rumen. Titi & Tabbaa (2004) manipulated the rumen microbes by feeding exogenous enzymes with cellulase activities to maximize fibre digestion in the rumen and Beauchemin *et al*. (2000) used exogenous fibrolytic enzymes to complete the activity of fibre digestion by rumen microbes. Exogenous enzymes with cellulase activity extracted from fungal strains have been utilized in enhancing degradation of structural carbohydrates (Nsereko *et al*., 2000).

Tricario & Dawson (1999) reported that when fescue hay was treated with fibrolytic enzyme high in xylanase and cellulase preparations, xylanase addition improved carbohydrate utilization and in *vitro* rumen fibre digestion. Zinn & Salinas (1999) observed increased ruminal NDF digestion (23%) and total tract N digestion (5%) when a direct fed rumen-stable fibrolytic enzyme supplement was added to the feed. Improvements in fibre digestion increased the quantity of microbial crude protein available to the animal when exogenous enzymes were supplemented in ruminant diets (Nsereko *et al*., 2002). Based on results obtained by these authors, it appears that the addition of exogenous fibrolytic enzymes from fungal extracts may be an effective means of improving cell wall degradation of high fibre forages, thereby increasing availability of nutrients (energy and protein) for growth of rumen microbes and for production by ruminants (Lewis *et al*. 1996).

The objective of this study was to evaluate the effects of exogenous fibrolytic enzyme treatments on *in vitro* DM and NDF degradability, and GP fermentation of mature forages

3.2 MATERIAL AND METHODS

The study was done at the Stellenbosch University, Western Cape Province, South Africa.

3.2.1 Experimental design

The experiment was a 3 x 3 factorial design. Two exogenous fibrolytic enzymes and a control (no enzyme) and three types of forage substrates were used as treatments.

3.2.2 Enzyme treatments

Two liquid preparations of exogenous fibrolytic enzymes, SU-ABO374 and CSIR ABO374, were supplied by the Microbiology Department (Stellenbosch University) and Council for Scientific Industrial Research (CSIR), respectively. The enzyme preparations and control (no enzyme) were used as treatments in assessing *in vitro* degradability of lucerne hay (LH), oat hay (OH) and wheat straw (WS). The two enzyme solutions were prepared from the same fungal extracts and containing a cocktail of fibrolytic exogenous enzyme activities. The major enzyme activities of the cocktail found were β-xylanase, β-endoglucanase, β-mannose and β-glucosidase (Microbiology Department, enzymology group Stellenbosch University, 2004). Both fungal β-xylanase and β-endoglucanase have been shown to improve ruminant feed digestibility (Beauchemin *et al.*, 2003). Level of exogenous enzymes (SU-ABO374 and CSIR-ABO374) application was measured in the previous study by (Goosen, 2004). The key enzyme profile of SU-ABO374 and CSIR-ABO374 enzymes supernatant was unidentified on the substrate it was cultivated from (wheat straw) by the manufactures. However, Cruywagen & Goosen (2004) reported improved growth rates and feed conversion ratios in growing lambs consuming a diet pre-treated with high and medium concentrations of exogenous enzyme ABO374. The enzyme solution SU-ABO374 was prepared on a small scale by the Microbiology Department and supplied fresh on a weekly basis. The CSIR-ABO374 enzyme solution was prepared on a larger scale by the CSIR. The CSIR enzyme solution contained a stabilizing agent.

3.2.3 *In vitro* rumen degradation

Forage sample preparations

The chemical compositions of the three forage substrates are presented Table 3.1. Substrate samples were milled through a 2 mm screen (Hammer Mill Ser. No. 372, Scientech RSA, Cape Town. RSA), and sieved with a mechanical shaker (Model Siemens Schuckert, J. Engelsman, Ludwigshafen, a. Rh., Germany) for 5 minutes using a 180 µm sieve to remove dust and extremely fine particles. Dacron bags (50 x 50 mm; Item #R510, ANKOM Technology, Fairport, New York, USA) were dried at 100°C overnight prior to use in order to determine the empty bag dry weights (W_1) before addition of the feed sample. Approximately 0.5 g of forage substrate (W_2) was weighed into each bag. Each forage substrate was weighed into 48 Dacron bags; additional 6 bags with substrate were added for sampling at time zero. The bags were heat sealed with an impulse bag sealer (Model #1920, Type: AIE-200, ANKOM Technology, Fairport, New York, USA). For each forage substrate (LH, OH, WS), eight bags were randomly selected and placed in each of the six DAISY digestion jars (2 L jars). Each digestion jar therefore contained 24 sample bags (ANKOM Technology, Fairport, New York, USA). The six jars were then grouped into 3 groups with two jars per group, which were labeled SU-ABO374, CSIR-ABO374 and Control. The composition of the forages is presented in Table 3.1.

Medium and enzyme preparations

A medium solution containing a buffer, macro- and micro-minerals, tryptose and rezasurin was prepared as described by Goering & Van Soest (1970). The buffer contained sodium bicarbonate and ammonium bicarbonate. The ammonium bicarbonate served as a source of nitrogen for rumen micro-organisms and tryptose provided nitrogen as peptides. Rezasurin was added to indicate the oxidation status of the medium. Jars containing the medium solution were placed in a water bath (39°C) and the headspace was gassed with carbon dioxide (CO_2) for one hour to reduce the pH to about 6.8. Approximately 1076 ml of medium was transferred to each digestion jar. Anaerobic conditions were maintained by gassing the headspace with CO_2.

Table 3.1 Chemical composition of the forages used in the evaluation of exogenous fibrolytic enzymes.

	Forages		
Item	**Lucerne hay**	**Oat hay**	**Wheat straw**
DM (g/kg)	909	892	928
OM (g/kg)	905	885	906
CP (g/kg)	185	75	63
NDF (g/kg OM)	513	686	767
ADF (g/kg DM)	381	430	548
ADL (g/kg DM)	95	70	90

DM = dry matter
OM = organic matter
CP = crude protein
NDF = neutral detergent fibre
ADF = acid detergent fibre
ADL = acid detergent lignin

The SU-ABO374 enzyme solution for the *in vitro* trial was prepared by transferring 1 ml fungal extract into a 100 ml volumetric flask and filling it up to the mark with distilled water. The CSIR-ABO374 enzyme was diluted by pipetting 1 ml fungal extract into a 250 ml volumetric flask and filling it up to the mark with distilled water. The difference in dilution levels was essential for SU-ABO374 and CSIR-ABO374 enzyme solutions, because the enzyme activity of CSIR-ABO374 was indicated to be 2.5 times that of SU-ABO374. The activities of the enzymes SU-ABO374 and CSIR-ABO374 were found to contain; β-xylanase, β-endoglucanase, β-mannose and β-glucosidase (Microbiology Department, enzymology group Stellenbosch University). From the diluted enzyme solutions, 6 ml was pipetted and added to each of the jars labeled SU-ABO374 and CSIR-ABO374. A similar procedure was used in dispensing distilled water to jars labeled as Control.

Reducing solution was prepared based on modifications by Van Soest & Robertson (1985). About 54 ml of reducing solution was added to the digestion jars. The jars were continually gassed with CO2 prior to and during the addition of reducing solution to maintain anaerobic conditions. The jars

were closed and placed into the DAISY^{II} incubator, which was pre-heated to 39°C, to equilibrate while rumen fluid was collected.

Rumen fluid collection and incubation

Rumen fluid was collected from two ruminally cannulated Döhne Merino whethers that received a diet of 50% lucerne and 50% wheat straw supplemented with a concentrate (500g/day). Rumen fluid was collected about 3 h after animals were fed in the morning. Rumen contents were removed by hand and squeezed through two layers of cheesecloth into pre-warmed thermo flasks for transport to the laboratory. The rumen fluid was blended in a Waring blender (Waring Products Division, New Hartford, USA) at low speed for 30 seconds and filtered through two layers of cheesecloth. Filtered rumen fluid was placed in a 39°C water bath and pH was recorded while gassing the headspace with CO_2. Two hundred and seventy ml of rumen fluid was added to each of the digestion jars. The head space of the digestion jars were gassed with CO_2, closed and placed in the DAISY^{II} incubator for 96 h. The experiment was repeated four times over a period of four weeks.

Dacron bag sampling and chemical analyses

Duplicate zero hour sampling bags for each substrate were soaked in three beakers containing approximately 150 ml of distilled water for 30 minutes. Beakers were labeled SU-ABO374, CSIR-ABO374 and Control. Each beaker contained 3g of sample (0.5g x 6) and therefore 1.5 ml of the appropriate enzyme solution was added per beaker. The bags were oven-dried at 105°C for DM determination and stored at room temperature until analyzed for NDF and OM.

During sampling, three bags (one of each substrate) were removed from each jar after 2, 4, 8, 12, 24, 48, 72 and 96 h of incubation. They were then thoroughly rinsed with cold tap water, allowed to dry at room temperature and placed in a conventional oven at 105°C overnight for complete drying. The dry samples were weighed to determine DM degradation.

Analytical DM content of the feed samples was determined by drying at 105°C overnight. Percentage dry matter contents of the feed used as substrate *(W_0)* was determined gravimetrically. Dry matter (DM) values were determined for each feed using the following equation: (sample DM = W_0/100 x W_2, where W_0 is a DM percentage of each substrate and W_2 is a substrate sample weight). Methods described by ANKOM Technology (ANKOM Technology, Fairport, New York, USA) were used to determine NDF, ADF, and ADL. To determine NDF, the residues were treated with ND solution and boiled using ANKOM^{200} Fiber Analyzer (ANKOM Technology, Fairport, New York, USA) for 75 minutes to remove microbial residual matter and any remaining soluble fractions. Approximately 20g of sodium sulphite was added to 2 L of ND solution to reduce nitrogenous contamination of fibre (Van Soest, 1994). The NDF residue was dried at 105°C in a conventional oven and dry weights were recorded. Disappearance of OM was determined by ashing bags with residual NDF at 500°C in a muffle furnace for 6 h to determine the ash content. The organic matter (OM) content was calculated as the difference between DM and ash contents. The NDF residue was expressed on an ash-free basis.

To determine acid detergent fibre (ADF), the feed samples (approximately 0.5g in a dacron bag) were treated with AD solution (20 g of cetyl trimethylammonium bromide was added to one liter 1N sulphuric acid) using ANKOM^{200} Fiber Analyzer (ANKOM Technology, Fairport, New York, USA) for one hour. The ADF residues (cellulose and lignin) were dried at 100°C overnight in a conventional oven and residual dry weights were recorded. Following ADF analysis, the residues were soaked in 72% sulphuric acid for three hours to determine acid detergent lignin (ADL). The lignin residues were washed with warm water and dried at 100°C overnight in a conventional oven and residual dry weights were recorded. The percentage ADF and ADL of feeds were calculated on a DM basis.

Data obtained from the *in vitro* degradability trial was fitted to the following non-linear model (Ørskov & McDonald, 1979):

$$P = a + b\,(1 - e^{-ct})$$

Where P = degradation at time t (%)

a = soluble and rapidly degradable fraction (%)

b = insoluble degradable fraction (%)

t = time (h)

c = rate at which b is degraded (%/h)

Effective degradation DM and NDF was calculated as: a + ((b*c)/(c+k)), where k was accepted to be 0.08.

In vitro gas production

Forage substrates (LH, OH and WS) were weighed into glass vials and analyzed based on the method of Theodorou *et al*. (1994). About 0.5 g of substrate was weighed accurately into 120 ml glass vials containing magnetic stir bars of known volume. Each treatment level was replicated 3 times.

The medium, enzyme solutions, reducing solution and rumen fluid used were prepared as described in section 3.2. About 40 ml of buffered medium, 2 ml reducing solution and 0.5 ml diluted enzyme solutions were added to each vial. Blanks vials, containing buffered medium, reducing solution and rumen fluid; and buffered medium, reducing solution, enzymes and rumen fluid were included to correct for gas release and fermentation residues resulting directly from the buffered solution and enzymes (Eun *et al*., 2007). Vials were capped with degreased rubber stoppers (Type 2048-11800, Bellco Glass, Vineland, NJ) and placed in a pre-warmed incubator at 39°C for the medium to equilibrate and be reduced. Rumen fluid was then added at 10 ml/vial.

The headspace of each vial was gassed with CO_2 while rumen fluid was being added. The vials were re-capped with rubber stoppers and fitted with crimp sealed caps to ensure that no gas could escape. The pressure caused by the insertion of the rubber stoppers was released by pushing a hypodermic needle (21G x 1.5 mm) through the rubber stopper and removing it again immediately. Atmospheric pressure thus represented the zero hour value. The vials were then placed in an incubator with magnetic stir plates to provide continuous mild agitation of the contents throughout the fermentation for 96 h. Temperature was maintained at 39^{o}C in a temperature controlled incubator.

In vitro gas production sampling and chemical analyses

Gas production was manually measured at 2, 4, 8, 12, 24, 48, 72 and 96 h using a digital pressure gauge (Model SDPG0015PG5, SenSym ICT, Honeywell Inc., Morris NJ) fitted with a 21 mm gauge needle. Accumulated gas was released after every measurement beyond 12 h of incubation by inserting a 21 mm gauge needle through the rubber stopper. After 96 h of incubation, fermentation was stopped by removing the rubber stoppers and placing the bottles on ice for approximately 15 minutes. The contents of the bottles were filtered under vacuum (No. 790999, Speed vac ED150, British, England) into previously weighed dacron bags. The filtered residues were semi-dried overnight at room temperature, after which the bags were heat-sealed. Bags were then placed in a force-draught oven and dried at 105°C, after which they were weighed and analyzed for NDF residue and ash content as described in section 3.4.

In vitro gas production values (ml/g OM) were fitted to the following non-linear model (Ørskov & McDonald, 1979):

$Y = b (1 - e^{-kt})$

Where Y = gas volume at time t (ml)

b = asymptotic gas production (ml/g OM)

t = time (h)

k = fractional rate of gas production (ml/h)

3.2.4 Statistical analysis

Data obtained from both the in vitro rumen degradation and GP trials were subjected to a two way ANOVA with the aid of Statistica 6.1 (2006). Differences between means were detected using a Bonferrori test. Significance was declared at $P < 0.05$. Curves for *in vitro* DM and NDF degradation and GP were plotted using Microsoft Excel (2003) software.

3.3 RESULTS AND DISCUSSION

3.3.1 *In vitro* rumen degradation

Effects of forages treated with exogenous fibrolytic enzymes on *in vitro* DM and NDF degradation (disregarding enzyme effect)

The effect of forage type (lucerne hay, oat hay and wheat straw) is presented in Table 3.2. The forages differed significantly in terms of DM and NDF degradation ($P<0.05$). Lucerne hay had the highest soluble fraction and wheat straw the lowest. Dry matter degradability for LH was 47.9%, 55.0% for OH and 52.8% for WS. Effective DM degradability (DMeff) was high for LH at 50.3% and low at 25.9% for WS. The fractional rate of DM degradation of LH was the highest, followed by oat hay and wheat straw. Effective NDF degradation (NDFeff) for LH was also high at 30.0% and WS had the least at 18.4%. A similar trend was reflected in the fraction rate of NDF degradation at 0.057%/h for LH which was almost 3 times higher than that of WS at 0.017%/h. Oat hay had higher NDF degradability at 77.6% than LH at 56.4%. The DMeff and NDFeff were low for OH and WS when compared with LH. The observed variation was mainly due to the differences in chemical composition and forage type (Table 3.1). Van Soest (1994) stated that DM and NDF degradability of forages is associated with chemical composition of substrates.

Effects of exogenous fibrolytic enzymes applied to forages on *in vitro* DM and NDF degradation (disregarding forage effect)

Results of exogenous enzyme application on *in vitro* degradation of forage are presented in Table 3.3. Application of exogenous enzymes did not have a significant effect on DM and NDF degradation of forages ($P>0.05$). Dry matter degradability of forages averaged at 52.0% with similar enzyme treatments. The NDF degradability was high and ranged between 66.7 and 71.8%, with SU-ABO374 showing the highest NDF degradability on forages, but showing smaller different responses when . The DMeff and NDFeff for forages were similar and averaged at 37.0% and 23.0%, respectively, with similar exogenous enzymes. Rate of DM degradation was similar

averaged at 0.043%/h and averaged at 0.031%/h for NDF degradation of forages, with similar enzyme treatments. Hristov *et al*. (2000) reported no effect of enzyme treatment on digestibility of

DM, CP, or NDF in cattle fed a barley grain-based diet. In agreement with the results reported from this study and the previous author, Wallace *et al*. (2001) also found that supplementation of enzymes did not increase digestibility of corn silage or grass silages. However, Feng *et al*. (1996) reported improved DM, NDF and ADF digestibility when fibrolytic enzymes were applied to grass hay before feeding to cattle.

Table 3.2 *In vitro* DM and NDF degradation parameter estimates (%) for different forages treated with exogenous fibrolytic enzymes (disregarding enzyme effects)

Parameter	Forages			SEm	P
	Lucerne hay	Oat hay	Wheat straw		
DM					
a	21.2[a]	14.2[b]	9.5[c]	0.269	<0.01
b	47.9[a]	55.0[b]	52.8[c]	1.514	<0.01
c	0.078[a]	0.028[b]	0.023[c]	0.0016	<0.01
DMeff	50.3[a]	33.7[b]	25.9[c]	0.449	<0.01
NDF					
a	0.00[a]	0.49[b]	0.09[c]	0.158	0.834
b	56.4[a]	77.6[b]	73.8[c]	3.387	<0.01
c	0.057[a]	0.019[b]	0.017[c]	0.0013	<0.01
NDFeff	30.0[a]	21.7[b]	18.4[c]	0.583	<0.01

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which DM and NDF is degraded (%/h)
DMeff= effective DM degradation (%)
NDFeff= effective NDF degradation (%)
SEm = standard error of means
[a, b, c] Means with different superscripts in rows differed significantly P<0.05

Table 3.3 *In vitro* DM and NDF degradation parameter estimates (%) for exogenous fibrolytic enzymes applied to forages (disregarding forage effects).

Parameter	Enzyme treatments			SEm	P
	SU-ABO374	CSIR-ABO374	Control (no enzyme)		
DM					
a	14.9	14.9	15.0	0.269	0.928
b	51.9	52.5	51.3	1.514	0.854
c	0.044	0.041	0.044	0.002	0.359
DMeff	36.8	36.4	36.7	0.450	0.816
NDF					
a	0.14	0.17	0.00	0.125	0.914
b	71.8	69.3	66.7	3.387	0.567
c	0.031	0.031	0.032	0.0013	0.791
NDFeff	23.2	23.5	23.6	0.583	0.884

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which DM and NDF is degraded (%/h)
DMeff= effective DM degradation (%)
NDFeff= effective NDF degradation (%)
SEm = standard error of means
Significance was declared at $P<0.05$

Effects of exogenous fibrolytic enzymes application on *in vitro* DM and NDF degradation for different forage substrates

Data of exogenous enzyme application on DM and NDF of different forages are presented in Table 3.4 and Table 3.5. Exogenous enzyme treatments did not have an effect on DM and NDF degradation of forages ($P>0.05$). Dry matter degradation was similar and averaged 48.5% for LH, 55.7% for OH and 52.7% for WS across enzymes. Effective DM (DMeff) was also not affected by enzyme treatment (within forages) and was high for LH, which averaged 50.3% and low for WS at 25.8% (Table 3.4). The rate of DM degradation within forages was similar with different enzyme treatments, with LC showing a high average rate of 0.08%/h, which was 4 times higher than that of WS at 0.02%/h.

Table 3.4 *In vitro* DM degradation parameter estimates (%) of different forages treated with exogenous fibrolytic enzymes.

Parameter	Enzyme treatment			SEm	P
	SU-ABO374	CSIR-ABO374	Control (no enzyme)		
Lucerne hay					
a	20.9	21.4	21.3	0.465	0.886
b	47.9	48.2	47.7	2.621	0.995
c	0.08	0.07	0.08	0.003	0.789
DMeff	50.4	50.2	50.3	0.778	0.991
Oat hay					
a	14.4	14.0	14.2	0.465	0.886
b	54.7	55.7	54.7	2.621	0.995
c	0.03	0.03	0.03	0.003	0.789
DMeff	34.1	33.3	33.7	0.778	0.991
Wheat straw					
a	9.5	9.3	9.6	0.465	0.886
b	53.3	53.5	51.4	2.621	0.995
c	0.02	0.02	0.02	0.003	0.789
DMeff	25.6	25.8	26.1	0.778	0.991

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which DM is degraded (%/h)
DMeff= effective DM degradation (%)
SEm = standard error of means
Significance was declared at $P<0.05$

Potential NDF degradability for LH improved with CSIR-ABO374 but not with SU-3ABO74 and a Control (Table 3.5). Neutral detergent fibre degradability for OH improved with both exogenous enzymes but decreased with a control. Enzyme treatments with WS, NDF degradability differed across enzyme treatments with SU-ABO374 indicating a higher NDF degradability for WS at 81.4% than CSIR-ABO374 at 71.8% and low for Control at 68.2%, however the response to enzymes treatment was not significantly effective. Effective NDF degradability (NDFeff) for different forages was not affected, but was high for LH averaged at 30.0% and low for WS at 18.3% across enzymes. Treatment had no effect on NDF degradation rate, but LH indicated a higher rate averaged at 0.056%/h and low for OH and WS at 0.017 %/h. Munn *et al*. (2002) reported

improvements on *in vitro* NDF and OM degradation of lucerne hay when exogenous enzyme (cellulose and hemicellulose complex) was applied at different concentration levels (0.05 vs. 0.50), but no effect on DM degradation. No effect results were also reported by these authors on degradability for wheat straw in which NDF degradability was not improved when the same exogenous enzymes (cellulose and hemicellulose complex) applied to lucern hay was applied to wheat straw at same concentration levels. Suzuki *et al*. (1994) used a fungal enzyme preparation in sheep fed hay or straw diet, and they reported that enzyme had no effects on DM or NDF digestibility.

Table 3.5 *In vitro* NDF degradation (%) parameter estimates of different forages treated with exogenous fibrolytic enzymes.

	Enzyme treatments				
Parameter	**SU-ABO374**	**CSIR-ABO374**	**Control (no enzyme)**	**SEm**	**P**
Lucerne hay					
a	0.00	0.11	0.03	0.146	0.894
b	55.8	57.3	55.9	5.867	0.786
c	0.057	0.056	0.057	0.002	0.779
NDFeff	29.7	30.4	29.9	1.009	0.810
Oat hay					
a	0.27	0.26	0.17	0.146	0.894
b	78.3	78.7	75.9	5.867	0.786
c	0.019	0.018	0.02	0.002	0.779
NDFeff	22.0	21.0	22.3	1.009	0.810
Wheat straw					
a	0.00	0.02	0.16	0.146	0.894
b	81.4	71.8	68.2	5.867	0.786
c	0.015	0.019	0.018	0.002	0.779
NDFeff	17.8	18.9	18.4	1.009	0.810

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which NDF is degraded (%/h)
NDFeff = effective NDF degradation (%)
SEm = standard error of means
Significance was declared at $P<0.05$

Degradation of forage DM and NDF is graphically illustrated in. Figures 3.1 to 3.3 (DM) and Figures 3.4 to 3.6 (NDF) The degradation curves are tightly overlaid, confirming the lack of treatment effects. Results indicate that exogenous enzyme application (SU-ABO374 and CSIR-ABO374) did not have a significant effect on DM and NDF degradation of forages. Based on the observation from the curves, these results conclude that the exogenous enzymes did not have an influence over a control on DM and NDF degradation for mature forages

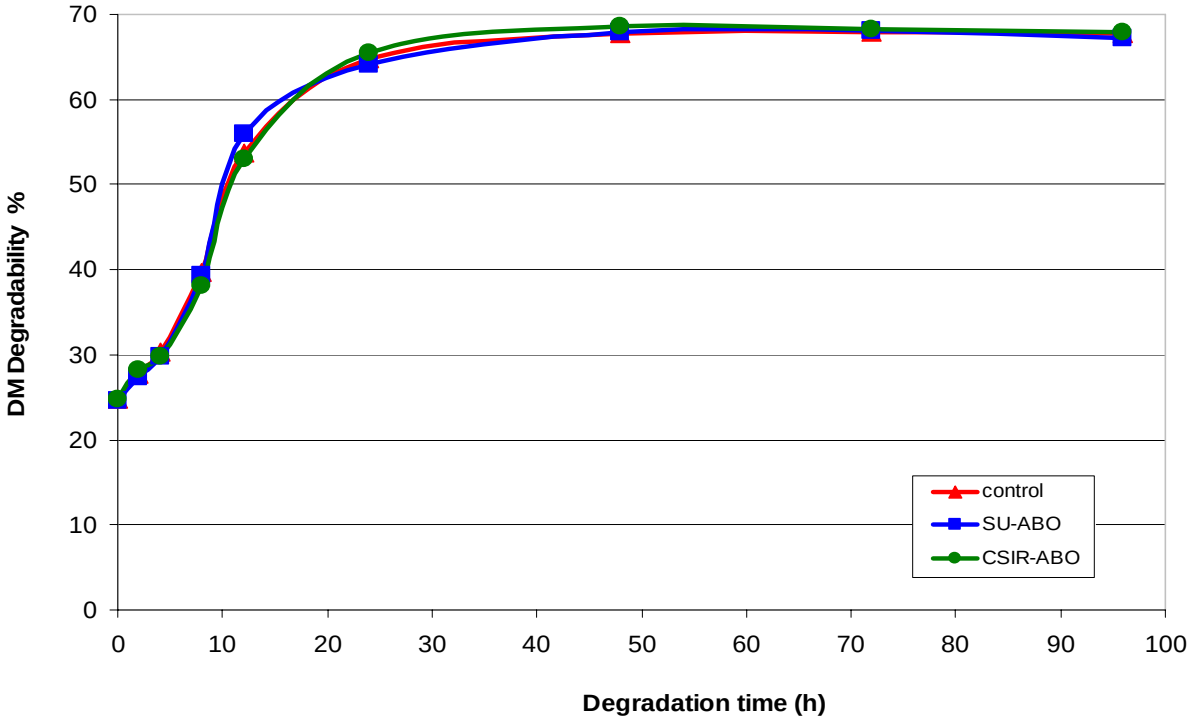

Figure 3.1 *In vitro* DM degradability (%) of lucerne hay incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

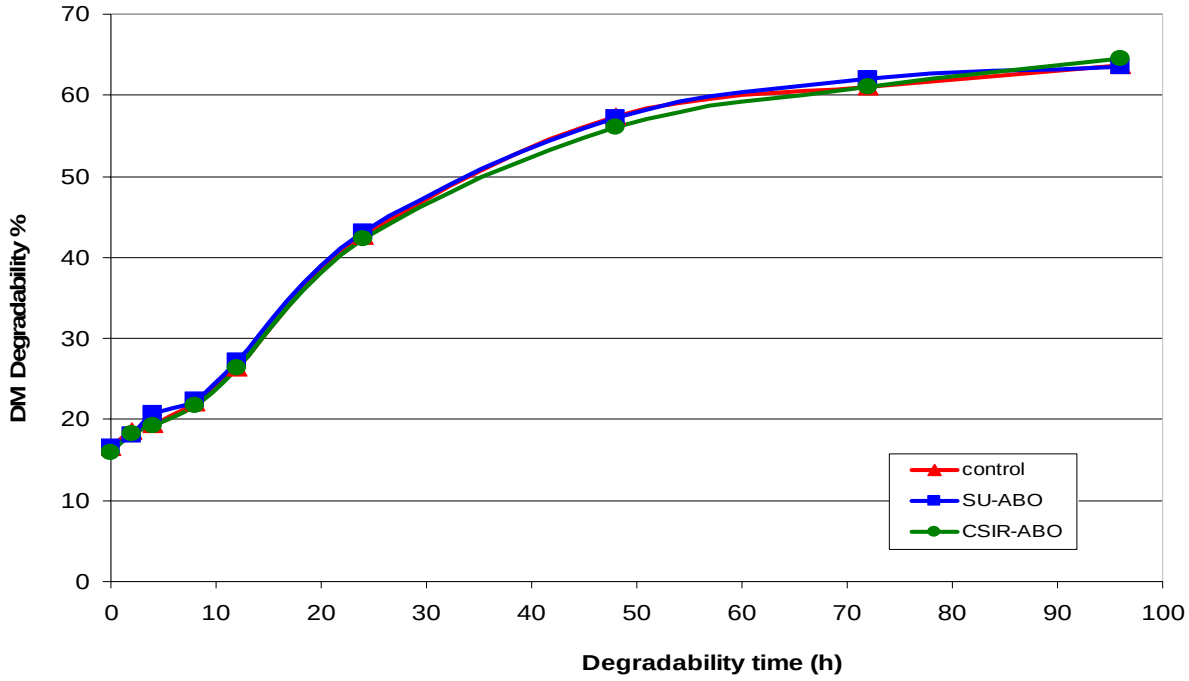

Figure 3.2 *In vitro* DM degradability (%) of oat hay incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

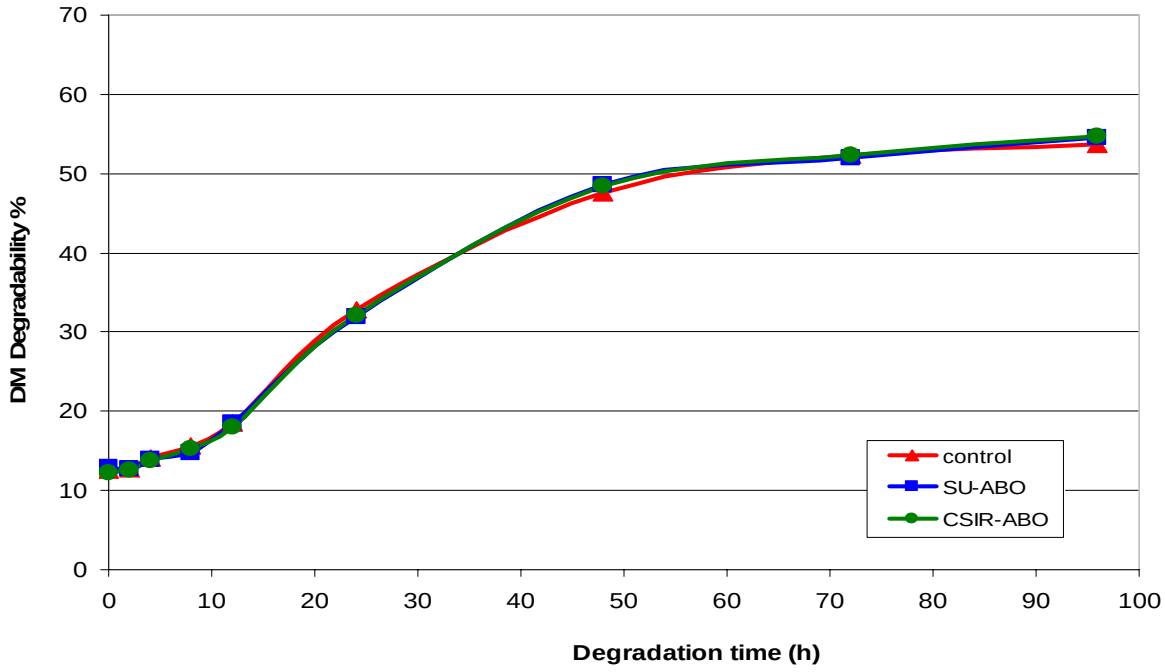

Figure 3.3 *In vitro* DM degradability (%) of wheat straw incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

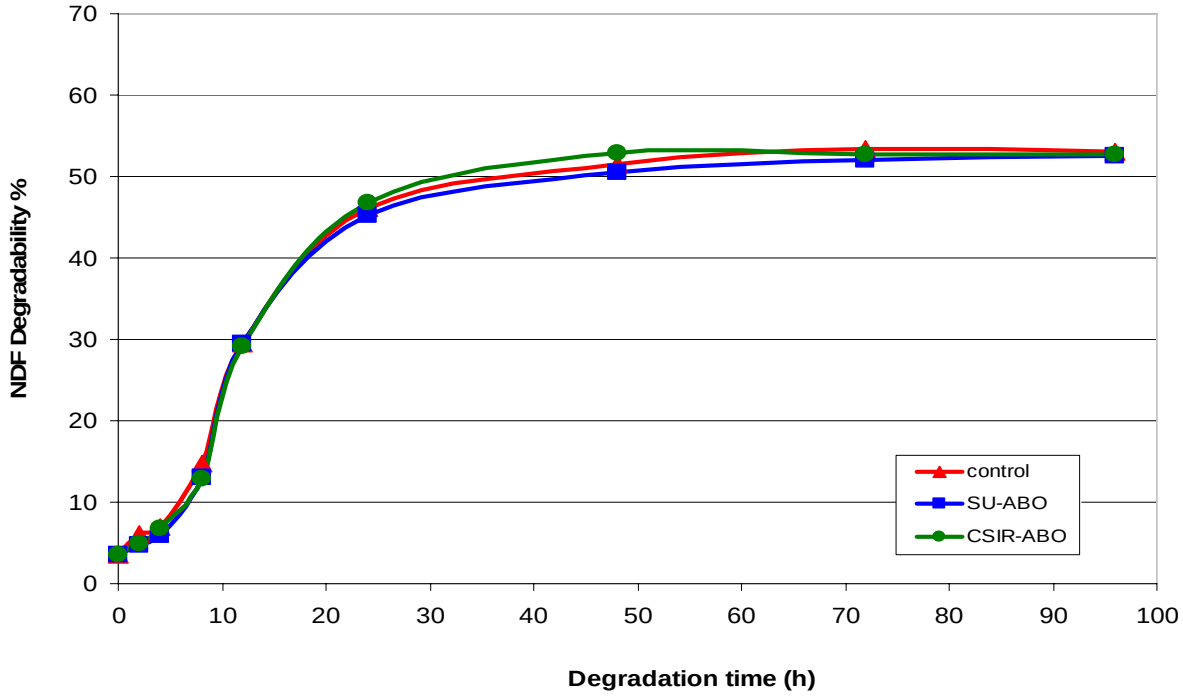

Figure 3.4 *In vitro* NDF degradability (%) of lucerne hay incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

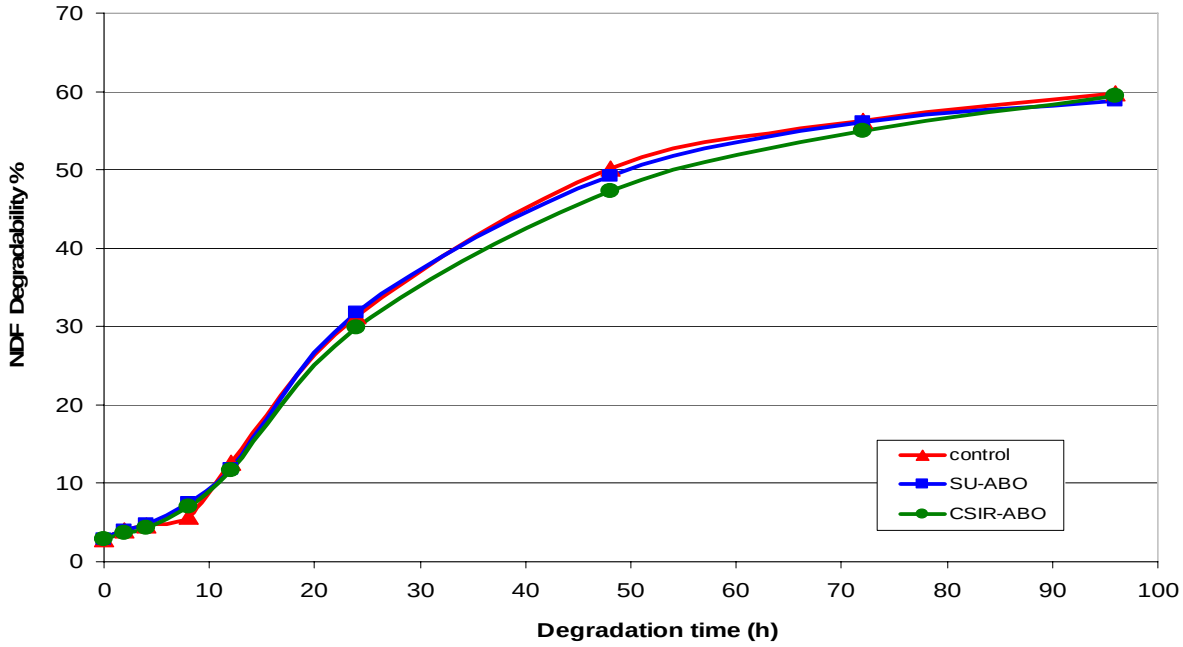

Figure 3.5 *In vitro* NDF degradability (%) of oat hay incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

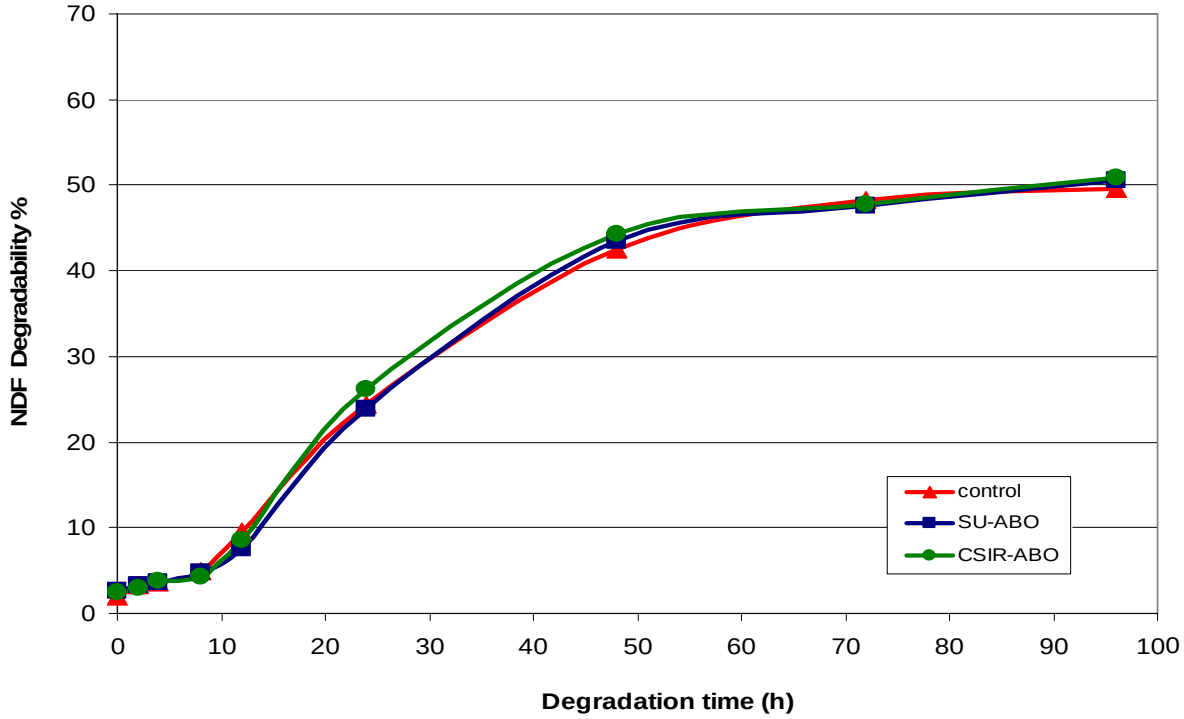

Figure 3.6 *In vitro* NDF degradability (%) of wheat straw incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

3.3.2 *In vitro* gas production

Effects of forages treated with exogenous fibrolytic enzymes on *in vitro* gas production (disregarding exogenous enzymes effect)

In vitro gas production estimates from 96 h incubation of forages are presented in Table 3.6. There were differences in gas production and rate of OM disappearance between forages ($P<0.05$). Gas production ranged between higher for WS at 489.9 ml/g OM and lower at 348.3 ml/g OM for LH. The fractional rate of GP was high at 0.03 ml/h for both LH and OH and low for WS at 0.01 ml/h. The amount of gas produced forages varied with forage type. The rate of at which the gas was produced was due to the variation in concentration and chemical composition of the forages (Table 3.1). Dolores *et al*., (2002) have stated that the amount of a gas produced by the forage varies with substrate type. The authors also stated that the rate at which gas is produced depend on the concentration and chemical composition of the feed. Wheat straw with higher fibre content produced more gas at low rate than lucern hay at higher rate with lower fibre content (Table 3.1).

Table 3.6 *In vitro* gas production (ml/g OM) parameter estimates for forages treated with exogenous fibrolytic enzymes (disregarding enzyme effects).

Parameter	Forages			SEm	P
	Lucerne hay	Oat hay	Wheat straw		
b	348.3[a]	376.9[b]	489.9[c]	22.8	<0.01
k	0.03[a]	0.03[a]	0.01[b]	0.004	<0.01

b = asymptotic gas volume (ml/g OM)
k = rate of gas production (ml/h)
SEm = standard error of means
[a, b, c] Means with different superscripts in rows differed significantly P<0.05

Effects of exogenous enzymes application on *in vitro* gas production of forages (disregarding forage effect)

The effect of different exogenous enzyme treatments on gas production of forages is presented in Table 3.7. Exogenous enzyme application did not significantly affect GP or the rate of GP across forages (P>0.05). Gas production from forages due to addition of exogenous enzymes ranged between 397.6 and 417.1 ml/g OM, with the highest gas production from SU-ABO374. The rate of GP was similar with enzyme treatments averaged at 0.026 ml/h. Not enough information from the literature

Table 3.7 *In vitro* gas production (ml/g OM) parameter estimates for exogenous enzymes applied to forages (disregarding forage effects).

Parameter	Enzyme treatments			SEm	P
	SU-ABO374	CSIR-ABO374	Control (no enzyme)		
b	417.1	397.6	400.4	22.8	0.810
k	0.025	0.026	0.026	0.004	0.962

b = asymptotic gas volume (ml/g OM)
k = rate of gas production (ml/h)
SEm = standard error of means
Significance was declared at P<0.05

Effects of exogenous fibrolytic enzymes application on *in vitro* gas production for different forages

Results showing the effect of different exogenous fibrolytic enzyme treatments on gas production of forages are presented in Table 3.8. Application of exogenous enzymes did not have an effect on gas production or the rate of OM disappearance of forages ($P>0.05$). Gas production was high for WS and ranged between 450.9 to 539.6 ml/g OM and low for LH, ranging between 341 and 354 ml/g OM, across enzyme treatments. The rate of GP was similar between treatments within forages and the average three times higher for LH and OH than for WS (across enzyme treatments). Wallace *et al*. (2001) also reported no improvement of exogenous enzymes with high cellulase activity and low xylanase activity on rate of gas production for grass silage.

Dry matter disappearance (DMdp) and NDF disappearance (NDFdp) of forages differed between enzyme treatments (Table 3.8). Effects differed with different forage substrates. Enzyme CSIR-ABO374 resulted in a lower DM disappearance than SU-ABO374 and Control for LH. The NDF disappearance in lucerne hay was the highest for SU-ABO374, followed by Control and CSIR-ABO374. In oat hay, both DM and NDF disappearance was the highest in the CSIR-ABO374 treatment, while the Control yielded the lowest values. The NDF degradability of oat hay was, however, the highest for the CSIR-ABO374 treatment, followed by Control. In wheat straw, the CSIR-treatment yielded the lowest DM degradability, but the highest NDF degradability. These interactions are difficult to explain, but may be related to a difference in the fibre matrix of the different forages. There is no clear pattern to be discerned regarding enzyme treatment on either DM or NDF degradability. However, the effect of enzymes treatment on NDF disappearance differed among forages as result of enzyme-feed specificity.

Table 3.8 *In vitro* gas production (ml/g OM) parameter estimates for different forages treated with exogenous enzymes.

Parameter	Treatment			SEm	P
	SU-ABO374	CSIR – ABO374	Control (no enzyme)		
Lucerne hay					
b (ml/g OM)	341.4	354.1	349.3	39.6	0.673
k (ml/h)	0.034	0.035	0.035	0.007	0.999
DMdp	65.7[a]	57.2[b]	66.5[a]	2.51	0.018
NDFdp	57.7[a]	46.9[b]	54.5[c]	2.52	<0.01
Oat hay					
b (ml/g OM)	370.3	387.8	372.6	39.6	0.673
k (ml/h)	0.033	0.035	0.034	0.007	0.999
DMdp	62.2[a]	65.5[b]	56.6[c]	2.51	0.018
NDFdp	45.6[a]	57.3[b]	53.4[c]	2.52	<0.01
Wheat straw					
b (ml/g OM)	539.6	450.9	479.4	39.6	0.673
k (ml/h)	0.009	0.011	0.009	0.007	0.999
DMdp	64.6[a]	58.9[b]	63.2[c]	2.51	0.018
NDFdp	51.4[a]	57.0[b]	45.1[c]	2.52	<0.01

b = asymptotic gas volume (ml/g OM)
k = rate of gas production (ml/h)
DMdp= dry matter disappearance (%)
NDFdp= neutral detergent fibre disappearance (%))
SEm = standard error of means
Significance was declared at P<0.05
[a, b, c]Means with different superscripts differed significantly P<0.05

The gas production curves of LH, OH and WS due to exogenous enzymes treatment are illustrated in Figures 3.7 to 3.9. No clear pattern can be observed for any of the treatments within forages. Between forages, it is clear that lucerne hay and oat hay fermented faster than wheat straw.

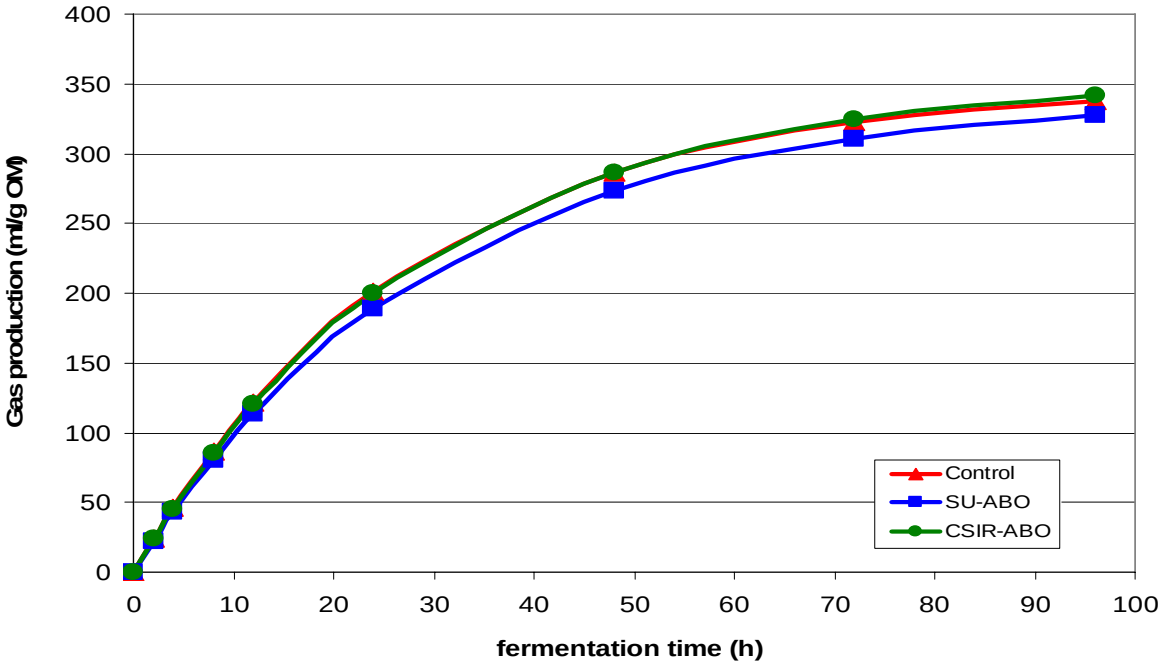

Figure 3.7 *In vitro* fermentation (ml/g OM) of lucerne hay, incubated with buffered medium and rumen fluid exogenous fibrolyic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

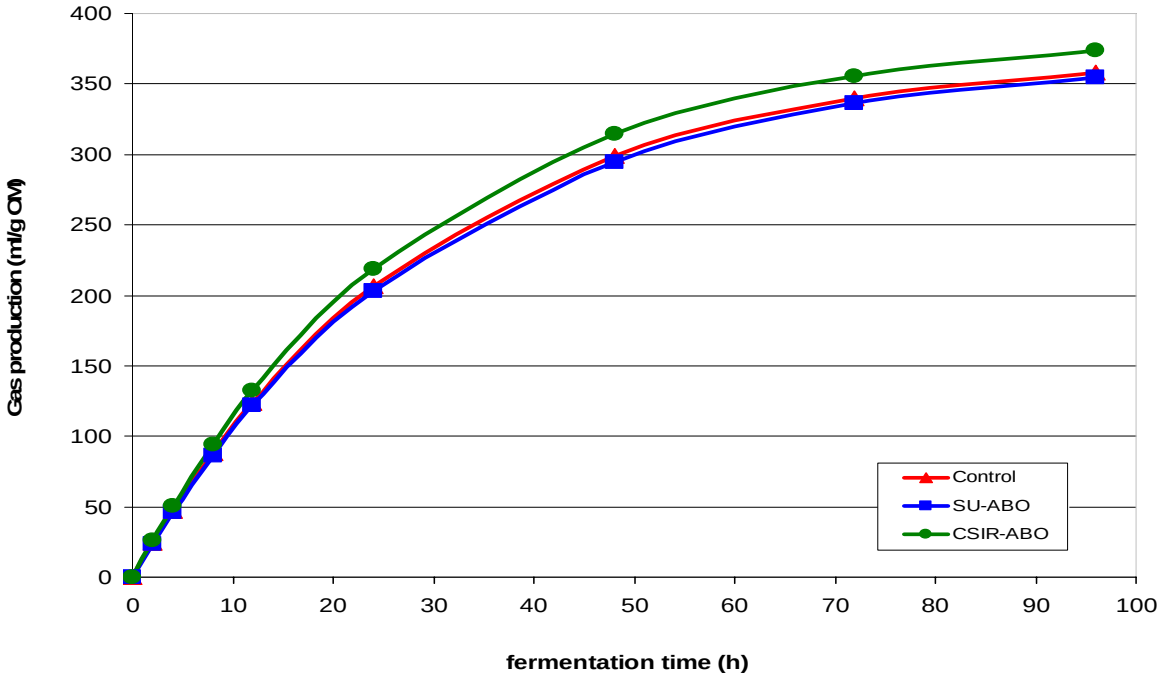

Figure 3.8 *In vitro* fermentation (ml/g OM) of oat hay incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

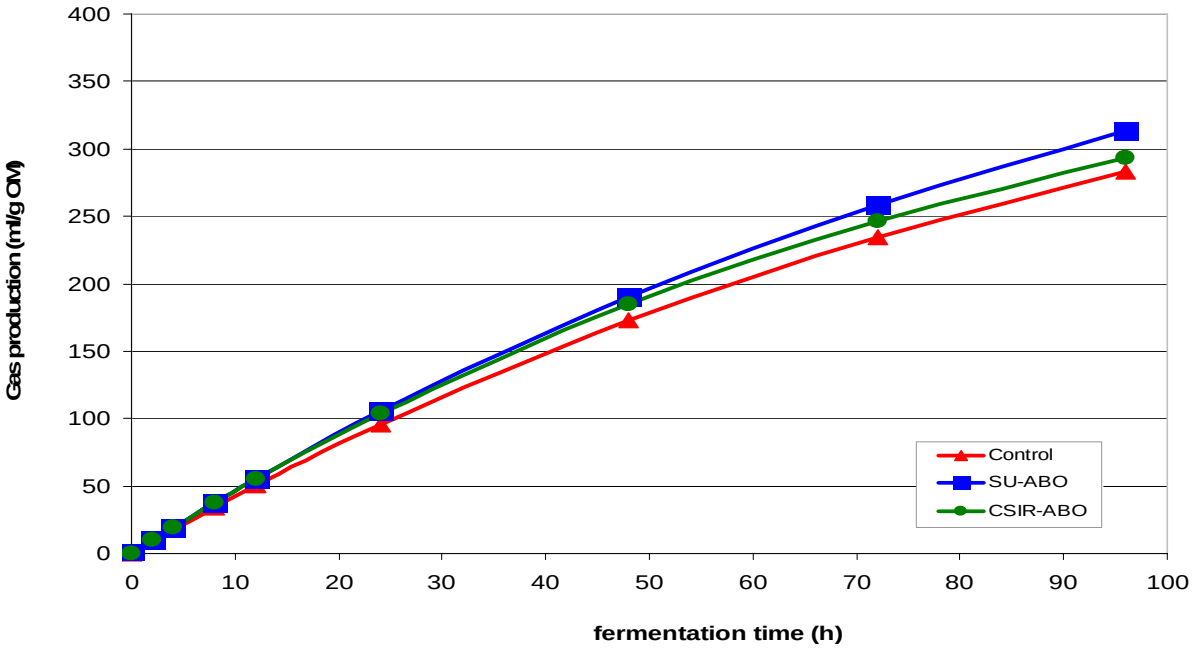

Figure 3.9 *In vitro* fermentation (ml/g OM) of wheat straw incubated with buffered medium and rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

Recent research has indicated that exogenous enzymes have the potential to increase forage degradation in the rumen (Beauchemin *et al*., 2003). However, the application rates at which the enzymes show optimal effects appear to depend on forage type (Beauchemin *et al*., 1995). The enzyme product used in the present study (SU-ABO374 and CSIR-ABO374) particularly contained xylanase activity, which has been shown to be positively correlated with the degradation of wheat straw in *vivo* (Cruywagen & Goosen, 2004). However, the results were negative when the same enzyme product was applied in the present study *in vitro.* Morgavi *et al.* (2000) used the ANKOM fermentation system to evaluate different modes of enzyme application. However, they found no effects when enzymes were added directly into the fermentation vessels,

Results suggest that lack of response from the present study on DM and NDF degradability and GP of mature forages may be due to exogenous enzymes not given enough time to interact with the substrates just before adding rumen fluid for incubation (pre-treatment effects). Beauchemin *et al*. (2003) have stated that exogenous enzymes are most effective when applied to feed prior to ingestion by cattle provided given more time to interact with a substrate, as it may cause release reducing sugars (Hristov *et al*., 1996), and cause partial solubilisation of NDF and ADF (Krause *et al*., 1998). In another study (Colombatto *et al*., 2003), observed increases in the rate of fermentation

and *in vitro* organic matter (OM) degradability when lucern stems were treated with commercial mixtures of xylanases and cellulases and allowed to interact for 20 h before inoculation with ruminal fluid. However, from this study it was not demonstrated whether these specific exogenous enzymes product requires an interaction time to show improvements in dry matter (DM) and fibre degradation. Stability of enzymes activities may have varied, as the enzyme was prepared freshly every week for four consecutive weeks from different batches. Differences in occasion for their production and may be temperature of the rumen environment may have caused them to be unstable.

Composition and activities of the enzymes product may also have influenced no effect results, due to lacking of enough information on the activities of the enzymes such as specific enzymes contained in the cocktail, optimum temperature and suitable pH due to confidentiality from the manufactures. It is not clear whether the effects of the exogenous enzymes were influenced by the environment (rumen pH and temperature) or other factors mentioned above. Level of application could also have been high or low for the substrates. Beauchemin *et al*., (2004) stated that the lack of response to enzyme supplementation may be due over or under supply of enzymes. Lewis *et al*. (1999) observed that a medium level of enzyme supplementation to forage produced more milk than a low or high level of application, and Beauchemin *et a*l. (2000) found that a high level of enzyme application to forages was less effective than a low level at increasing total tract digestibility.

Variability in response of exogenous enzymes (SU-ABO374 and CSIR- ABO374) for DM and NDF degradability and GP *in vitro* is associated with different feed types and chemical composition of forage substrate. Beauchemin *et al*. (1995) have stated that the effectiveness of a particular enzyme product depend upon the composition of the diet fed, because the composition and structure of plant cell wall is variable among forages (Aman, 1993). However, other factors such as substrate type, temperature provided, target substrate may also have influenced no effects results.

3.4 CONCLUSION

Treatment of lucerne hay, oat hay and wheat straw with the exogenous fibrolytic enzymes SU-ABO374 and CSIR-ABO374 did not appear to affect DM and NDF degradation as measured with the *in vitro* nylon bag technique. Enzyme treatments did not have an effect on the fermentation

characteristics of forages as measured with the *in vitro* gas production method. When the residues of the gas production trials were taken as an estimate of degradation over 96 hours, treatments had different effects and the interactions that were observed complicated interpretation. No clear pattern could be observed. In the current trial, it was concluded that the enzymes SU-ABO374 and CSIR-ABO374 had no clear effect on DM or NDF degradation or fermentation characteristics. Lucerne hay degraded at a higher rate than oat hay, which again degraded faster than wheat straw, regardless of enzyme treatment. Effective degradation was higher for lucerne hay than for oat hay, which was higher than that of wheat straw.

The lack of response may have been due to factors such as pre- treatment effects, reduced stability and enzymes activities, optimum temperature and pH of the enzymes.

3. 5 REFERENCES

Aman, P., 1993. Composition and structure of cell wall polysaccharides in forages. In: forage cell wall structure and digestibility. Jung, H.G., Buxton, D.R., Hatfield, R.D. & Ralph, (Eds.). ASA,CSSA,SSSA.Madison, WI. pp. 183-199.

ANKOM, 1998. Procedures for *in vitro* true digestibility using the DAISY^{II} incubator. URL Address: www.ankom.com.

Beauchemin, K.A., Rode, L.M. & Sewalt, V.J.H., 1995. Fibrolytic enzymes increase fiber digestibility and growth rate of steers fed dry forages. Can. J. Anim. Sci. 75, 641-644.

Beauchemin, K.A., Rode L.M., Maekawa, M., Morgavi, D.P. & Kanpen, R., 2000. Evaluation of non-starch polysaccharidase feed enzyme in dairy cows diets. J. Dairy Sci. 83, 543-553.

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(E. Suppl. 2): E37–E47.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z. & Rode, L.M., 2004. Mode of action of exogenous cell wall degrading enzymes for ruminants. Can. J. Anim. Sci. 84, 13-22.

Colombatto, D., & Beauchemin, K.A., 2003. A proposed methodology to standardize the determination of enzymic activities present in enzyme additives used in ruminant diets. Can. J. Anim. Sci. 83, 559-568.

Cruywagen, C.W. & Goosen, L., 2004. Effect of an exogenous fibrolytic enzyme on growth rate, feed intake and feed conversion ratio in growing lambs. S. Afr. J. Anim. Sci. 34 (Suppl. 2), 71-73.

Dolores M.M., Hernádez, F., Madrid, J & Teruel., A.M., 2002. Feeding value, *in vitro* digestibility and *in vitro* gas productions of different by-products for ruminant nutrition. J. Sci. Food Agric. 82, 567-572.

Eun, J.S, Beauchemin, K.A, & Schulze, H., 2007. Use of an *in vitro* fermentation bioassay to evaluate improvements in degradation of lucerne hay due to exogenous feed enzymes. Anim. Feed. Sci. Technol. 135(3-4), 315-328.

Feng, P., Hunt, C.W., Pritchard, G.T. & Julien, W.E., 1996. Effect of enzyme preparations on in situ and *in vitro* degradation and in vivo digestive characteristics of mature cool-season grass forage in beef steers. J. Anim. Sci. 74, 1349-1357.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J. & Carro, M.D., 2007. In press: Effects of exogenous fibrolytic enzymes on *in vitro* rumen fermentation of substrates with different forage: concentrate ratios. Anim Feed Sci. Techno., doi:10-1016/j.animfeedscience.2007.06.013.

Goering, H.K. & Van Soest, P.J., 1970. Forage fiber analyses (Apparatus, reagents, procedures and some applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC. USA.

Goosen, L., 2004. The effect of an exogenous fibrolytic enzyme on forage digestibility parameters. M.Sc. (Agric) thesis, University of Stellenbosch, Stellenbosch, South Africa.

Hristov, A. N., McAllister, T. A. & Cheng K. J., 2000. Intraruminal supplementation with increasing levels of exogenous polysaccharide-degrading enzymes: Effects on nutrient digestion in cattle fed a barley grain diet. J. Anim. Sci. 78:477-487.

Krause, M., Beauchemin, K.A., Rode L.M., Farr, B.I. & Nørgaard, P., 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Anim. Sci. 76, 2912-2920.

Lewis, G.E., Hunt, C.W., Sanchez, W.K., Treacher, R., Pritchard, G.T. & Feng, P., 1996. Effect of direct-fed fibrolytic enzymes on the digestive characteristics of a forage-based diet fed to beef steers. J. Anim. Sci. 74, 3020-3028.

Lewis, G. E., W. K. Sanchez, C. W. Hunt, M. A. Guy, G. T. Pritchard, B. I. Swanson, and R. J. Treacher. 1999. Effect of direct-fed fibrolytic enzymes on the lactation performance of dairy cows. J. Dairy Sci. 82:611–617

McAllister, T.A., Hristov, K.A., Beauchemin, K.A., Rode, L.M. & Cheng, K.J., 2001. Enzymes in ruminants diets In: Enzymes in farm animal nutrition. Bedford, M.R. & Partridge, G.G. (Eds.), CAB international, Wallingford., UK, pp. 273-298.

Mertens, D.R., 1997. Creating systems for meeting fiber requirements of dairy cows. J. Dairy Sci. 80, 1463-1481.

Morgavi, D.P., Wuerfel R.L., Nsereko, V.L., Beauchemin, K.A. & Rode, L.M., 2000. Effect of enzyme feed additives and method of application on *in vitro* feed digestibility, J. Dairy Sci. 83 (Suppl. 1), p. 291 (Abstr.).

Munn, V.A., Loëst, C.A., Mathis, C.P., Petersen, M.K., Endecott, R.L., Powers, T., & Waterman, R.C., 2002. Effects of enzyme feed additive on rumen degradability of mature forage *in vitro*. Proceedings, Western section, New Mexico state University, Las Cruces, NM. A. Soc. Anim. Sci. 53, 1-2.

Nsereko, V.L., Morgavi, D.P., Beauchemin, K.A. & McAllister, T.A., 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfafa hay fiber by mixed rumen microorganisms *in vitro*. Anim. Feed Sci. Technol. 88,153-170.

Nsereko, V.L., Beauchemin, K.A., Morgavi, D.P., Rode, L.M., Furtado, A.F., McAllister, T.A., Iwaasa, A.D., Wang, Y. & Yang, W.Z., 2002. Effect of a fibrolytic enzyme preparation from *Trichoderma longibrachiatum* on the rumen microbial population of dairy cows. Can. J. Microbiol. 48, 14-20.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric Sci., Camb. 92, 499-503.

Phipps, R.H., Sutton, J.D., & Bhat, M.K., 2002. Are enzymes useful in ruminant diets? In: Proceedings of British Society of Animal Science, Penicuik, UK. Br. Soc. Anim. Sci., pp. 246-247.

Statistica 6.1., 2006. StatSoft, Inc., US.

Suzuki, Y., J. Brinkmann and H.J. Abel, 1994. Effect of manioc meal and a cellulolytic enzyme preparation on digestibility and rumen fermentation in sheep. In: Gieseck, D. (Ed.) Proc. Soc. Nutr. Physiol. Frankfurt, Germany, p. 23.

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. Technol. 48, 185-197.

Titi, H.H. & Tabbaa, M.J., 2004. Efficacy of exogenous cellulose on digestibility in lambs and growth of dairy calves. Livestock Prod. Sci. 87, 207-214.

Tricario, J.M. & Dawson, K.A., 1999. Effects of defined xylanase and cellulase enzyme preparations on digestive processes of rumen microbial cultures. J. Dairy Sci. 77(Suppl. 1), 252 (Abstr).

Van Soest, P.J. & Robertson, J.B., 1985. Analysis of forages and fibrous foods. A laboratory manual for animal Science, Cornell University, Ithaca, NY, p. 613.

Van Soest, P.J., 1994. Nutritional ecology of the ruminant. 2nd edition. Cornell University Press, Ithaca, NY.

Wallace, R.J., Wallace, S.J.A., McKain, N., Nsereko, V.L., & Hartnell, G.F., 2001. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed rumen microorganisms in vitro. J. Anim. Sci. 79, 1905-1916.

Zinn, R.A., & Salinas, J., 1999. Influence of Fibrozyme on digestive function and growth performance of feedlot steers fed a 78% concentrate growing diet. Proc. Alltech 15th Annual Symposium. Biotechnology in the Feed Industry. Nottingham University Press, pp. 313-319.

CHAPTER 4

The effect of exogenous fibrolytic enzymes application on *in vitro* NDF degradation and gas production of mixed forage substrates with three levels of concentrate inclusion

Abstract

In vitro studies were conducted to evaluate the effect of exogenous fibrolytic enzymes on in vitro dry matter (DM) and neutral detergent fibre (NDF) degradation, and gas production (GP) of mixed concentrate and forage substrates. Two enzyme cocktails, SU-ABO374 and CSIR-ABO374, were cultivated in fungi on a local substrate (wheat straw) by the Microbiology Department (Stellenbosch University) and the CSIR, respectively. These, as well as a control treatment (no enzymes), were applied to three substrate mixtures differing in concentrate: forage ratio, viz. 80:20 (High Concentrate, HC), 50:50 (Medium Concentrate, MC) and 20:80 (Low Concentrate, LC). The forage component consisted of equal parts of lucerne hay, oat hay and wheat straw. Rumen fluid was collected from two ruminally cannulated Döhne Merino whethers fed a 50:50 mix of lucerne hay and wheat straw supplemented with a concentrate (500g/day). For the DM and NDF degradability trials, 500 mg of the appropriate substrate mixes were weighed into 50 x 50 mm dacron bags which were incubated anaerobically at 39ºC in 1.4L of a rumen liquid inoculated buffered medium in 2L fermentation jars. Bags from all treatments were removed after 2, 4, 8, 12, 24, 48, 72 and 96 h of incubation. For the gas production determinations, 500 mg of the appropriate substrates were weighed into 120 ml glass vials which were incubated for 96 h in 40 ml inoculated medium to which 0.5 ml of the respective enzyme solutions were added. Gas pressure was recorded manually with a digital pressure gauge after 2, 4, 8, 12, 24, 48, 72 and 96 h and pressure was converted to volume with a predetermined regression. The 96 h substrate residues were washed, dried, weighed and analyzed for NDF and OM. Enzyme treatments did not affect DM and NDF degradation or GP of the different concentrate: forage mixes. Although there were differences between the different concentrate: forage mixes in terms of NDF degradation and gas production, the enzyme treatments had no effect.

(*Key words*: Fibrolytic enzymes, forages, NDF degradability, gas production)

4.1 INTRODUCTION

Availability of ruminant products such as meat and milk is limited by plant nutrient supply. Accessibility of plant nutrients by fibre digesting microbes in the rumen largely depends on plant physical characteristics such as the structure and composition, as well as post harvest feed processing (Selinger *et al*., 1996). Piwonka & Firkins (1993) stated that digestion of plant cell wall in the rumen provides most of the energy for ruminants. Lactating dairy cows require an adequate amount of forage to maintain proper rumen function and fermentation for maximum production (Mertens, 1997). However, inclusion of moderate to high concentrations of grain in diets usually results in reduced fibre digestion in the rumen compared with high forage diets (Piwonka & Firkins, 1993). Shriver *et al*. (1986) stated that low pH (< 6.0) from fermentation of highly fermentable carbohydrates such as starch decreases growth of cellulolytic bacteria and hence, fibre digestion.

Exogenous fibrolytic enzymes containing xylanase and cellulose activities have been shown to enhance feed utilization thereby increasing fibre digestion by ruminants (Beauchemin *et al*., 1999). These fibrolytic enzymes have been used in high grain diets for dairy cows (Yang *et al*., 1999), and feedlot cattle (Beauchemin *et al*., 1997; Krause *et al*., 1998) to improve the digestibility of fibre in grain diets. Beauchemin & Rode (1997) reported improved fibre degradation of the fibrous cell wall of barley diets when exogenous enzymes with high xylanase and low cellulase activities were assessed *in vitro*. However, the researchers did not find improvements with corn based diets. Hristov *et al*. (1996) also reported that cellulase and xylanase preparations enhanced sugar release from barley diets but not corn diets when incubated *in vitro*. Improvements in DM, OM, NDF, and acid detergent fibre (ADF) degradability of a concentrate based diet were reported by Titi & Tabbaa (2004) when the diet was supplemented with exogenous enzymes containing cellulase activities. Krause *et al*. (1998) stated that exogenous enzymes may help overcome the depression in fibre digestion that occurs due to low ruminal pH when feeding high grain diets to high producing ruminants, whilst maintaining the productive performance.

Exogenous enzyme preparations have been evaluated primarily on the basis of their capacity to degrade plant cell walls of high fibrous diets. Due to enzyme specificity, there is high variability in their impact on different feed components. Cocktails of exogenous enzyme activities may provide better opportunities for improving digestion of structural carbohydrates in mixed substrate diets.

The objective of this study was to evaluate the effect of two cocktails of exogenous fibrolytic enzyme extracts (SU-ABO374 and CSIR-ABO374) on *in vitro* DM and NDF degradation, and GP of mixed forage substrates containing three levels of concentrate.

4.2 MATERIALS AND METHODS

The study was done at Stellenbosch University, Western Cape Province, South Africa.

4.2.1 Experimental design

The experiment was a 3 x 3 factorial design. Two exogenous enzymes and a control (no enzyme) were used as treatments and three concentrate-forage mixes with different levels of concentrates were used as substrates.

4.2.2 Enzyme treatments

Two liquid preparations of exogenous fibrolytic enzymes, SU-ABO374 and CSIR ABO374, were supplied by the Microbiology Department (Stellenbosch University) and Council for Scientific Industrial Research (CSIR), respectively. The enzyme preparations and control (no enzyme) were used as treatments in assessing *in vitro* degradability of lucerne hay (LH), oat hay (OH) and wheat straw (WS). The two enzyme solutions were prepared from the same fungal extracts and containing a cocktail of fibrolytic exogenous enzyme activities. The major enzyme activities of the cocktail found were β-xylanase, β-endoglucanase, β-mannose and β-glucosidase (Microbiology Department, enzymology group Stellenbosch University, 2004). Both fungal β-xylanase and β-endoglucanase have been shown to improve ruminant feed digestibility (Beauchemin *et al*., 2003). The application level for exogenous enzymes (SU-ABO374 and CSIR-ABO374) was measured in the previous study by (Goosen, 2004), but no information on the application level for concentrate diets. The key enzyme profile in the SU-ABO374 and CSIR-ABO374 enzymes supernatant was unidentified on the substrate it was cultivated from (wheat straw) by the manufactures. The enzyme solution SU-ABO374 was prepared on a small scale by the Microbiology Department and supplied fresh on a

weekly basis. The CSIR-ABO374 enzyme solution was prepared on a larger scale by the CSIR. The CSIR enzyme solution contained a stabilizing agent.

4.2.3 *In vitro* rumen degradation

Sample preparations

Three diets containing 80%, 50% and 20% of concentrate were used for enzyme addition evaluation. The balance of the diets consisted of equal parts of lucerne hay, oat hay and wheat straw. Ingredient and chemical composition of the diets are shown in Table 4.1. The concentrate diets were formulated to be iso-nitrogenous but varied in energy levels, using Microsoft Excel, (2003) software. The ingredients were milled through a 2 mm screen (Hammer Mill Ser. No. 372, Scientech, Cape Town, RSA) and sieved with a mechanical shaker (Model Siemens Schuckert, J. Engelsman, Ludwigshafen, a. Rh., Germany) through a 180 µm sieve for 5 minutes to eliminate the dust and extremely fine particles. Dacron bags (50 x 50 mm; Item #R510, 5 x 5 cm bags, ANKOM Technology, Fairport, New York, USA) were dried at 100°C overnight and weighed.

Approximately 0.5 g of diet substrate was weighed into each bag. For each of the concentrate diet substrates, 48 samples were weighed Dacron bags; 6 bags with substrate were included for sampling at time zero. The bags were heat sealed with an impulse bag sealer (Model #1920, Type: AIE-200, ANKOM Technology, Fairport, New York, USA). For each concentrate diet (80%, 50% and 20%), 8 bags were randomly selected and placed in each of the six DAISY digestion jars (2 L jars). Each digestion jar therefore contained 24 sample bags. The six jars were then grouped into 3 groups with two jars per group, which were labeled SU-ABO374, CSIR-ABO374 and Control.

Materials and methods for medium and enzyme preparations, rumen fluid collection and incubation, dacron bag sampling and chemical analysis were the same as that described in Chapter 3 (3.2.3).

Table 4.1 Ingredient and chemical composition of three concentrate diets used in the evaluation of exogenous fibrolytic enzymes

	Concentrate levels of diets		
Ingredients	**HC (80%)**	**MC (50%)**	**LC (20%)**
Physical composition		**% of diet DM**	
Wheat straw	20.1	49.8	79.8
Oat hay	20.1	49.8	79.8
Lucerne hay	20.1	49.8	79.8
Maize meal	198.0	111.0	24.0
Soya bean oil cake meal	31.2	30.0	30.0
Urea	3.0	3.0	2.7
Salt	1.8	1.8	1.5
Feed lime	5.4	4.5	2.1
Min/vit premix	0.3	0.3	0.3
Chemical Composition			
DM (g/kg)	874	876	883
OM (g/kg)	938	922	910
CP (g/kg)	164	170	163
NDF (g/kg OM)	206	372	540
ADF (g/kg DM)	133	252	383
ADL(g/kg DM)	28	49	68.6

DM = dry matter
OM = organic matter
CP = crude protein
NDF = neutral detergent fibre
ADF = acid detergent fibre
ADL = acid detergent lignin

Data obtained from the *in vitro* degradability trial was fitted to the following non-linear model (Ørskov & McDonald, 1979):

$P = a + b (1 - e^{-ct})$

Where P = degradation at time t (%)

a = soluble and rapidly degradable fraction (%)

b = insoluble degradable fraction (%)

t = time (h)

c = rate at which b is degraded (%/h)

Effective DM and NDF degradation was calculated as: a + ((b*c)/(c+k)), where k was accepted to be 0.08.

In vitro gas production

Gas production for the respective concentrate diets followed same sample preparations, materials and methods for medium and enzyme preparations, rumen fluid collection and incubation, gas production sampling and chemical analysis as that described in Chapter 3 (3.2.3 *in vitro* gas production). Accumulated gas was also manually released by inserting a 21 mm gauge needle at different time intervals (4, 8, 12 and 24 h) after measuring gas to prevent the gas from being reabsorbed into the fermentation solution.

In vitro gas production values (ml/g OM) were fitted to the following non-linear model (Ørskov & McDonald, 1979):

$Y = b (1 - e^{-kt})$

Where Y = gas volume at time t (ml)

b = asymptotic gas production (ml/g OM)

t = time (h)

k = fractional rate of gas production (ml/h)

4.2.4 Statistical analysis

Data were analyzed using two way ANOVA procedures of Statistica 6.1 (2006). Differences between means were detected using Bonferrori test. GP fermentation followed the same procedures as the in vitro rumen degradation. Significance was declared at $P < 0.05$. Curves for in vitro DM and NDF degradation and GP were plotted using Microsoft Excel (2003) software.

4.3 RESULTS AND DISCUSSION

4.3.1 *In vitro* rumen degradation

Effects of concentrate diets treated with exogenous fibrolytic enzymes on *in vitro* DM and NDF degradation (disregarding enzyme effect)

Dry matter and NDF degradation for concentrate diets (regardless enzyme treatment) are presented in Table 4.2. The concentrate diets differed significantly ($P < 0.05$) in DM and NDF degradation. The HC diet had the highest DM degradation at 71.2% and LC diet the lowest at 53.8%. The rate of DM degradation for HC was also higher at 0.065%/h, while that of LC was lower at 0.048%/h. Degradation of NDF was also higher for HC (74.5%) than for LC (62.8%). Although HC had the highest DM degradation, MC was degraded at almost the same rate as HC. There was no difference between diets regarding the rate of NDF degradability, but HC was degraded at a higher rate 0.037%/h than MC and LC.

Effective DM (DMeff) ranged higher for HC (50.5%) and lower at 35.5% for LC. Effective NDF (NDFeff) degradation values were also higher for HC than MC and MC higher than LC (Table 4.2). Generally, effective degradation values were low for the HC treatment which would be associated with the source of a soluble fraction and structural carbohydrates (NDF) from forages used in formulating the diets. Based on the concentrate level in the diet, it was assumed that the rapidly fermented fraction would have resulted in a reduction in fibre digestion because of high proportion of soluble carbohydrates from HC and visa versa with LC. The difference in DM and

NDF degradation for concentrate diets was mainly due to differences in concentration and chemical composition, and forage: concentrate ratio between diets (Table 4.1).

Table 4.2 *In vitro* DM and NDF degradation (%) parameter estimates for concentrate diets treated with exogenous fibrolytic enzyme (disregarding enzyme effects).

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Parameter	Substrates			SEm	P
	High concentrate	**Medium concentrate**	**Low concentrate**		
DM					
a	18.5[a]	15.5[b]	15.3[b]	0.651	<0.01
b	71.2[a]	63.4[b]	53.8[c]	0.679	<0.01
c	0.065[a]	0.061[a]	0.048[b]	0.003	<0.01
DMeff	50.5[a]	42.7[b]	35.5[c]	0.597	<0.01
NDF					
a	10.6[a]	1.9[b]	1.7$_c$	0.481	<0.01
b	74.5[a]	68.1[b]	62.8[c]	2.502	<0.01
c	0.037	0.035	0.034	0.003	0.667
NDFeff	32.8[a]	22.4[b]	20.2[c]	0.624	<0.01

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which DM and NDF is degraded (%/h)
DMeff= effective DM degradation (%)
NDFeff= effective NDF degradation (%)
SEm = standard error of means
Significance was declared at P<0.05
[a, b, c,] Means with different letters within rows differed significantly P<0.05

Effects of exogenous enzymes application on *in vitro* DM and NDF degradation of concentrate diets (disregarding the concentrate diets effect)

Application effects of exogenous enzymes (regardless diet composition) on DM and NDF degradation of the different concentrate diets are presented in Table 4.3. Exogenous fibrolytic enzymes did not have an effect on DM and NDF degradation of the different diets. Potential DM degradability of concentrate diets was similar and averaged 62.8%, while potential NDF degradability averaged 68.5%. Effective DM degradability and effective NDF degradability averaged 42.9% and 25.1%, respectively. The rate of DM degradability for concentrate diets was similar averaged at 0.057%/h with enzyme treatments.

Table 4.3 *In vitro* DM and NDF degradation (%) parameter estimates for exogenous fibrolytic enzymes applied to concentrate diets (disregarding the concentrate diet effects).

Parameter	Enzyme treatments			SEm	P
	SU- ABO374	**CSIR-ABO374**	**Control (No enzyme)**		
DM					
a	16.3	16.3	16.6	0.651	0.948
b	62.4	62.9	63.1	0.679	0.783
c	0.058	0.059	0.056	0.003	0.623
DMeff	42.7	43.3	42.7	0.597	0.679
NDF					
a	5.3	4.1	4.8	0.481	0.248
b	71.6	65.8	68.0	2.502	0.268
c	0.033	0.039	0.034	0.003	0.241
NDFeff	24.9	25.5	25.1	0.624	0.765

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which DM and NDF is degraded (%/h)
DMeff= effective DM degradation (%)
NDFeff= effective NDF degradation (%)
SEm = standard error of means
Significance was declared at $P < 0.05$

Effects of exogenous fibrolytic enzyme application on *in vitro* DM and NDF degradation for diets with different concentrate levels

The effect of exogenous fibrolytic enzyme treatments on DM and NDF degradation for different concentrate diets are presented in Tables 4.4 and 4.5. Exogenous enzyme treatments did not have an effect on DM degradation for concentrate diets ($P > 0.05$), however the enzyme CSIR-ABO374 showed improvement on DM degradation of HC but not SU-ABO374 (Table 4.4). Both MC and LC diets responses on DM degradation were similar due to exogenous enzymes treatment. Effective DM (DMeff) was similar averaged, fairly high for HC at 50.5% and low at 35.4% for LC. Degradation for NDF on different concentrate diets differed with enzyme treatments. Improvement was observed for different diets with different enzyme treatment i.e. SU-ABO374 was indicated improvement for HC, but reduced with CSIR-ABO374; however the response was not significantly

effective. The rate of DM and NDF degradation was similar for the different enzyme treatments within concentrate diets. Effective NDF degradability (NDFeff) averaged 32.8% for HC and 20.3% for LC. These results on NDF degradation indicate that the effects of enzymes on fibre content are influenced by the nature of the diet to which they are applied to.

Table 4.4 *In vitro* DM degradation (%) parameter estimates for diets with different concentrate levels treated with exogenous fibrolytic enzymes.

	Enzyme treatments				
Parameter	**SU-ABO374**	**CSIR-ABO374**	**Control (no enzyme)**	**SEm**	**P**
High concentrate					
a	18.9	18.3	18.3	1.127	0.899
b	69.9	71.5	72.4	1.176	0.717
c	0.06	0.07	0.07	0.005	0.430
DMeff	49.3	51.5	50.7	1.035	0.599
Medium concentrate					
a	14.7	15.8	15.8	1.127	0.899
b	63.8	63.2	63.3	1.176	0.717
c	0.07	0.06	0.06	0.005	0.430
DMeff	43.5	42.7	41.9	1.035	0.599
Low concentrate					
a	15.4	14.9	15.6	1.127	0.899
b	53.7	54.1	53.5	1.176	0.717
c	0.05	0.05	0.05	0.005	0.430
DMeff	35.3	35.7	35.4	1.035	0.599

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which DM is degraded (%/h)
DMeff= effective DM degradation (%)
SEm = standard error of means
Significance was declared at $P < 0.05$

Table 4.5 *In vitro* NDF degradation (%) parameter estimates for diets with different concentrate levels treated with of exogenous fibrolytic enzymes.

Parameter	Enzyme treatments			SEm	P
	SU-ABO374	CSIR-ABO374	Control (no enzyme)		
High concentrate					
a	11.6	9.9	10.3	0.834	0.719
b	83.5	68.1	71.9	4.334	0.380
c	0.030	0.044	0.038	0.004	0.239
NDFeff	31.3	33.8	33.5	1.080	0.436
Medium concentrate					
a	1.7	1.5	2.7	0.834	0.719
b	67.2	67.8	69.4	4.334	0.380
c	0.040	0.036	0.031	0.04	0.239
NDFeff	23.3	22.2	21.8	1.080	0.436
Low concentrate					
a	2.4	0.9	1.6	0.834	0.719
b	64.2	61.6	62.7	4.334	0.380
c	0.031	0.038	0.034	0.004	0.239
NDFeff	20.2	20.6	19.9	1.080	0.436

a = soluble and rapidly degradable fraction (%)
b = insoluble degradable fraction (%)
c = rate at which NDF is degraded (%/h)
NDFeff= effective NDF degradation (%)
SEm = standard error of means
Significance was declared at $P < 0.05$

Degradation of concentrate diets DM and NDF is graphically illustrated in Figures 4.1 to 4.6. The degradation curves (curves for DM 4.1 to 4.3 and NDF 4.4 to 4.5) are tightly overlaid confirming lack of treatment effects. Results indicate that exogenous enzyme application (SU-ABO374 and CSIR-ABO374) did not have a significant effect on DM and NDF degradation of the different concentrate diets.

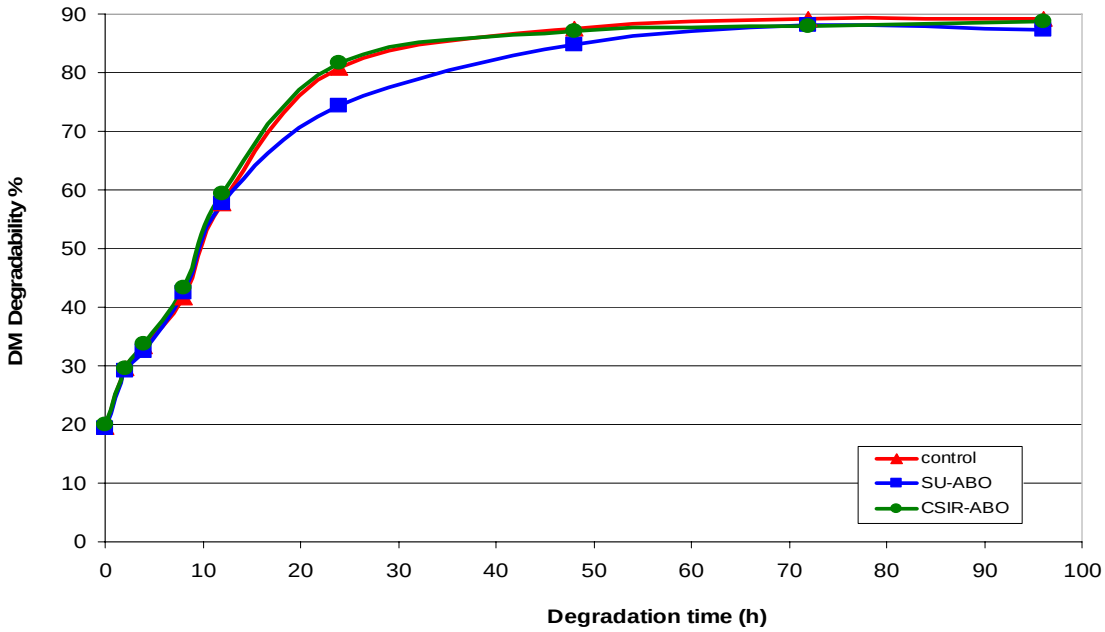

Figure 4.1 *In vitro* DM degradability (%) of the high concentrate diet (80% concentrate) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

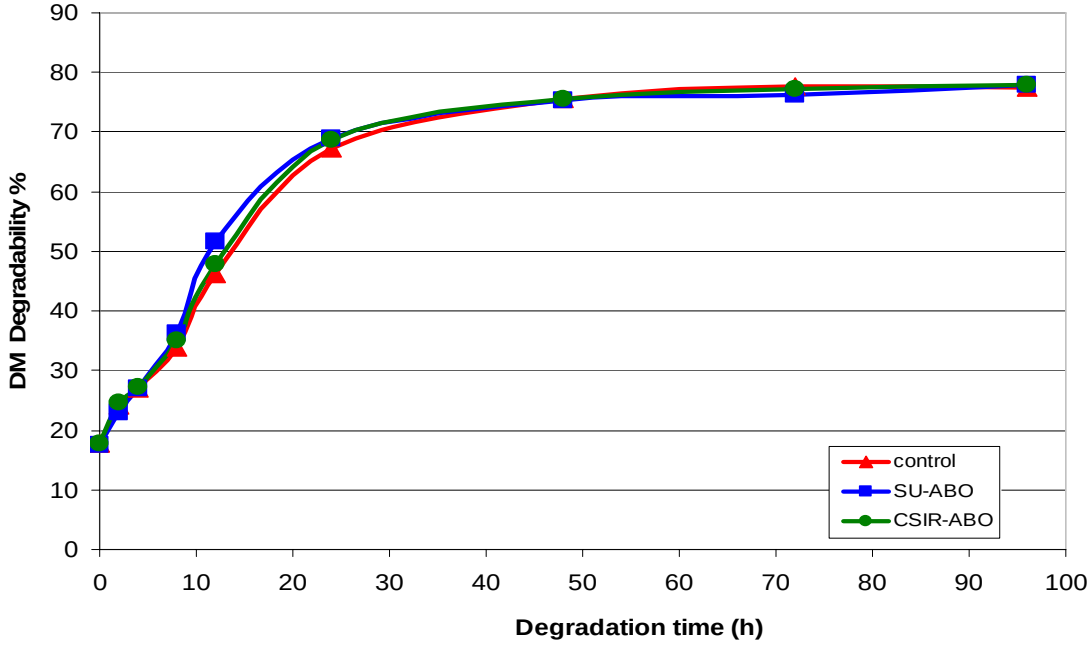

Figure 4.2 *In vitro* DM degradability (%) of medium concentrate diet (50% concentrate) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes (SU-ABO374, CSIR-ABO374) for 96 h.

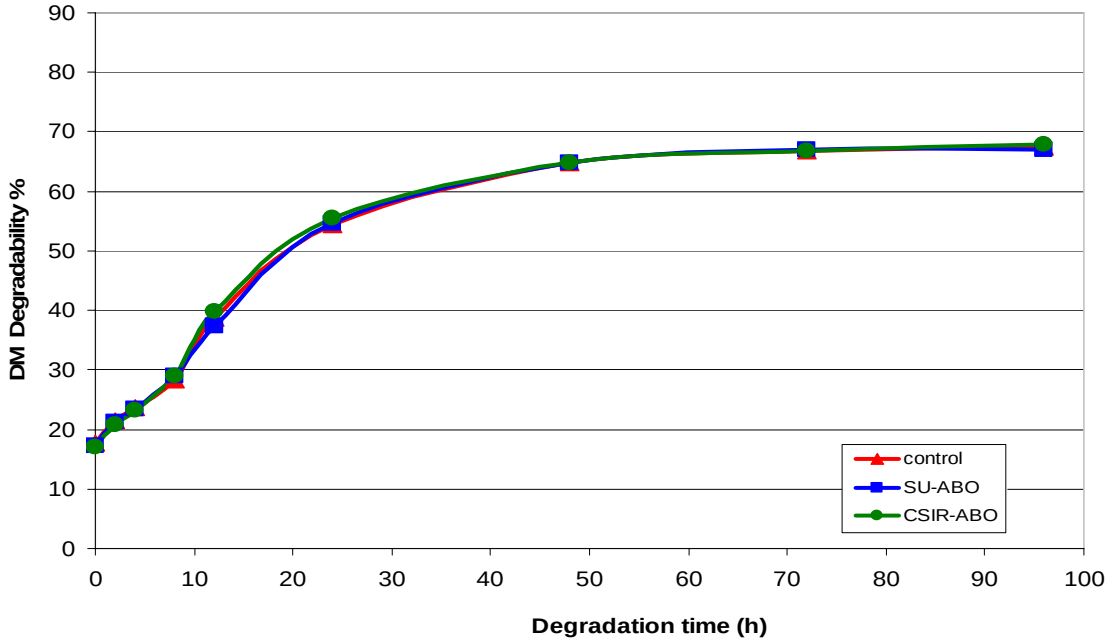

Figure 4.3 *In vitro* DM degradability (%) of low concentrate diet (20% concentrate) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

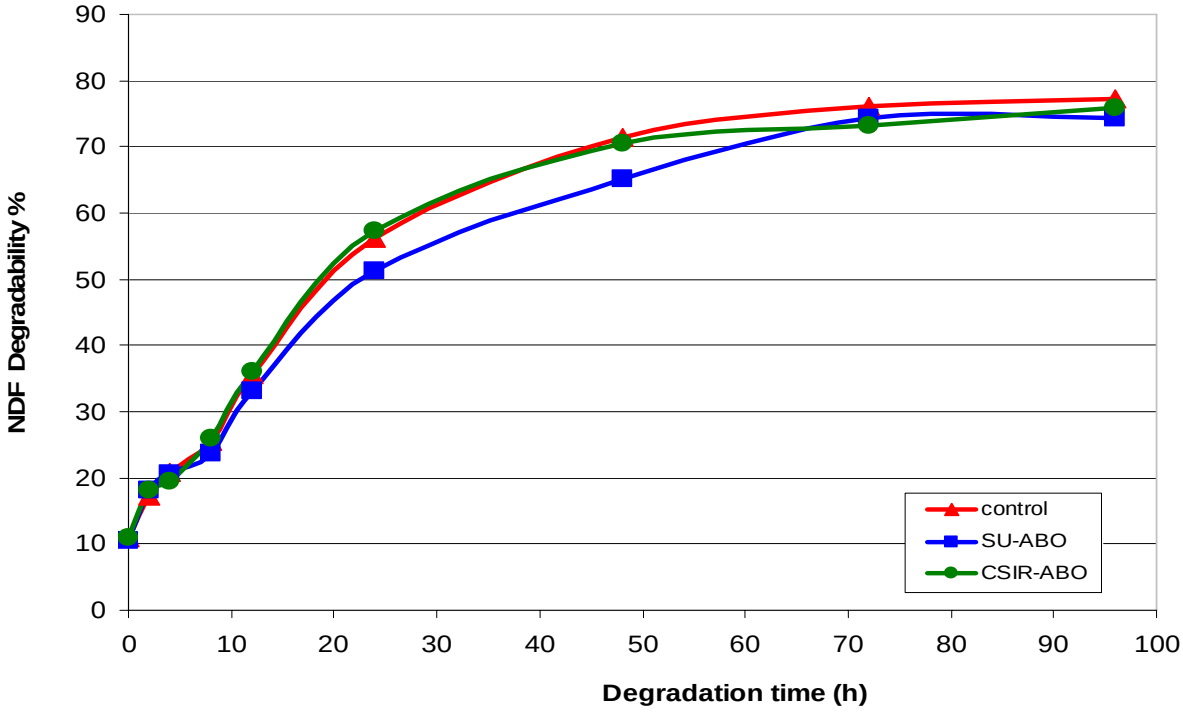

Figure 4.4 *In vitro* NDF degradability (%) of high concentrate diet (80% concentrate) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

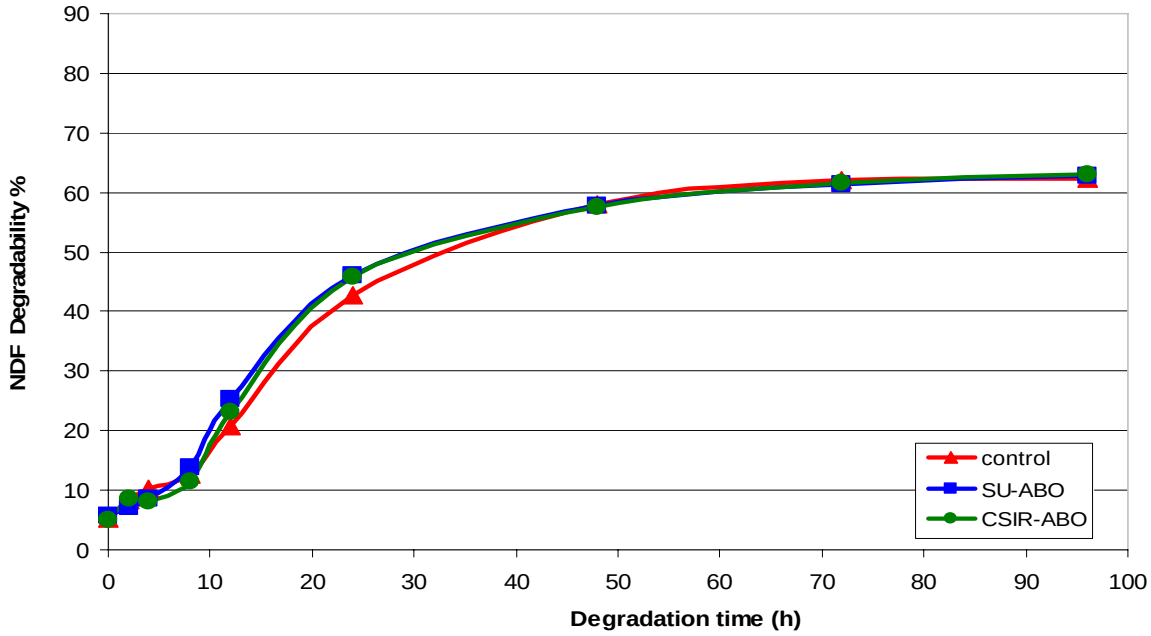

Figure 4.5 *In vitro* NDF degradability (%) of medium concentrate diet (50% concentrate) incubated with buffered medium, rumen fluid and exogenous fibroltyic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

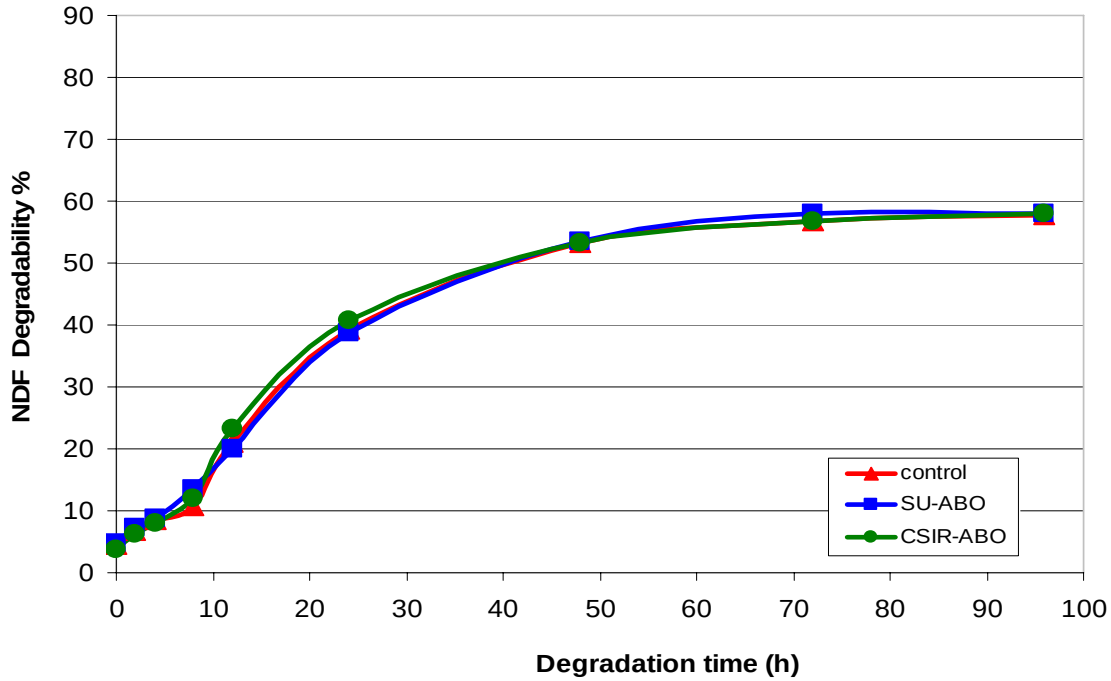

Figure 4.6 *In vitro* NDF degradability (%) of low concentrate diet (20% concentrate) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

The exogenous fibrolytic enzymes were added on the speculation that the enzymes would enhance the utilization of the diet and degradation of the fibre portion of the diets (i.e. 20% forage for HC, 50% forage for MC and 80% forage for LC). From the results obtained from this study, exogenous fibrolytic enzymes did not improve the DM and NDF degradation of the diets. These results support are the conclusions of Beauchemin & Rode (1997) who reported no improvements in fibre digestion of corn based diets treated with exogenous fibrolytic enzymes containing either low xylanase and high cellulase or high xylanase and low cellulase activities. Vicini *et al*. (2003) also reported no effect on DM and OM digestion of high forage and low concentrate diet (60:50 ratio) treated with exogenous enzymes. Giraldo *et al*. (2007b) observed no effect of enzymes containing xylanase activities on NDF and hemicelullose degradation for medium forage substrate (50%) on a 24 h *in vitro* incubation. The authors also reported no effect for low forage substrate (30%) pre-treated with the same exogenous enzymes. However, Yang *et al*. (2000) reported improvements on DM digestibility when exogenous enzymes were applied to a diet with 38% forage. Based on responses reported by these authors, their results support Beauchemin *et al*. (2003) who stated that the effectiveness of the enzymes varies with the component and proportion (forage: concentrate ratio) of the diet to which the enzyme is added.

4.3.2 *In vitro* gas production

Effects of concentrate diet substrates treated with exogenous fibrolytic enzymes on *in vitro* gas production (disregarding enzyme effects)

In vitro gas production estimates of concentrate diets are presented in Table 4.6. Gas production and fractional rate of gas production differed ($P<0.05$) between substrates. The HC diet had the highest estimate for gas production and a higher rate of fermentation when compared with MC and LC diets. Gas production for HC was high at 439.5 ml/g OM and low at 304.9 ml/g OM for LC. These results indicate that the estimates of gas produced by each concentrate substrate were influenced by forage: concentrate ratio. The differences were mainly as a result of variability in composition and fibre content (NDF, ADF and ADL) of the concentrate diets (Table 4.1). Rymer *et al*. (2005) reported that high fibre feeds produce more gas than low fibre feeds. Wheat straw with the highest NDF content had the highest gas production.

Table 4.6 *In vitro* gas production (ml/g OM) parameter estimates for diets with different levels of concentrate treated with exogenous fibrolytic enzymes (disregarding enzyme effects).

Parameter	Substrates (diets)			SEm	P
	High concentrate	Medium concentrate	Low concentrate		
b	439.52[a]	370.2[b]	304.9[c]	7.419	<0.01
k	0.07[a]	0.06[b]	0.05[c]	0.002	<0.01

b = asymptotic gas volume (ml/g OM)
k = rate of gas production (ml/h)
SEm = standard error of means
[a, b, c,] Means with different letters within rows differed significantly P < 0.05

Effect of exogenous fibrolytic enzyme application on gas production for concentrate diets (disregarding concentrate diet effects)

Results of exogenous fibrolytic enzyme treatments on gas production for concentrate diets are presented in Table 4.7. Exogenous enzymes did not improve the gas production or the rate of fermentation for concentrate diets (P>0.05). Average gas production for exogenous enzyme treatments was 372 ml/g OM. The rate of GP was similar for all treatments at 0.06 ml/h.

Table 4.7 *In vitro* gas production (ml/g OM) estimates for exogenous fibrolytic enzyme treatments applied to concentrate diets (disregarding concentrate diet effects).

Parameter	Enzyme treatments			SEm	P
	SU-ABO374	CSIR-ABO374	Control (no enzyme)		
b	376.8	367.0	370.8	7.419	0.649
k	0.06	0.06	0.06	0.002	0.464

b = asymptotic gas volume (ml/g OM)
k = rate of gas production (ml/h)
SEm = standard error of means
Significance was declared at P < 0.05

Effect of exogenous fibrolytic enzyme application on gas production for diets with different concentrate levels

The effect of exogenous enzyme application on diets containing different concentrate levels is presented in Table 4.8. Exogenous enzyme treatments did not have an effect (P>0.05) on gas production or the fractional rate for concentrate diets. The gas production ranged between 428 and 447 ml/g OM for HC, 356 and 383 ml/g OM for MC, and 298 and 317 ml/g OM for LC across all enzyme treatments, with HC (low fibre 20%) indicating higher gas production from exogenous enzymes treatment than LC (high fibre 80%). The average rate of GP did not differ between treatments and was 7.3 ml/h for HC, 6.7 ml/h for MC and 5 ml/h for LC with all enzyme treatments. These results support the conclusions by Wallace *et al*. (2001) who reported no improvement in rate of gas production for corn silage treated with exogenous enzymes with high xylanase activity and low cellulase activity.

Dry matter disappearance (DMdp) and NDF disappearance (NDFdp) of the concentrate diet substrates differed with enzyme treatments (P < 0.05). In the HC and MC substrates, DMdp was highest for the CSIR-ABO374 treatment and lowest for the Control, while the result was opposite for the LC substrate. The NDFdp pattern differed from the DMdp pattern; for the HC substrates, the CSIR-ABO374 treatment resulted in the highest NDFdp, while for the MC substrates SU-ABO374 resulted in the highest NDFdp. For the LC substrate, both SU-ABO374 and Control resulted in higher NDFdp values than CSIR-ABO374.

Table 4.8 The effect of exogenous fibrolytic enzyme application on gas production (ml/g OM) estimates for diets with different concentrate levels.

Parameter	Enzyme treatments			SEm	P
	SU-ABO374	CSIR-ABO374	Control (no enzyme)		
High concentrate					
b (ml/g OM)	447.2	427.8	443.5	12.850	0.439
k (ml/h)	0.07	0.07	0.08	0.004	0.998
DMdp	81.1[a]	83.6[b]	71.7[c]	3.857	0.035
NDFdp	52.9[a]	55.3[b]	51.4[c]	1.856	0.017
Medium concentrate					
b (ml/g OM)	383.1	356.4	371.1	12.850	0.439
k (ml/h)	0.06	0.07	0.07	0.004	0.998
DMdp	77.6[a]	83.9[b]	73.5c	3.857	0.035
NDFdp	54.9[a]	44.9[b]	51.3c	1.856	0.017
Low concentrate					
b (ml/g OM)	300.0	316.8	297.7	12.850	0.439
k (ml/h)	0.05	0.05	0.05	0.004	0.998
DMdp	79.4[a]	72.9[b]	84.5[c]	3.857	0.035
NDFdp	54.9[a]	49.4[b]	54.9[a]	1.856	0.017

b = gas volume (ml/g OM)
k = rate of gas production (ml/h)
DMdp= dry matter disappearance (%)
NDFdp= neutral detergent fibre disappearance (%)
SEm = standard error of means
[a, b, c] Means with the same letter within rows did not differ significantly $P < 0.05$

In vitro gas production incubation curves for the different concentrate diets are illustrated in Figures 4.7 to 4.9. The gas production curves run parallel but were closely related. No clear pattern can be observed for any of the treatments within concentrate diets. Between concentrate diets, it is clear that HC and MC fermented faster than LC.

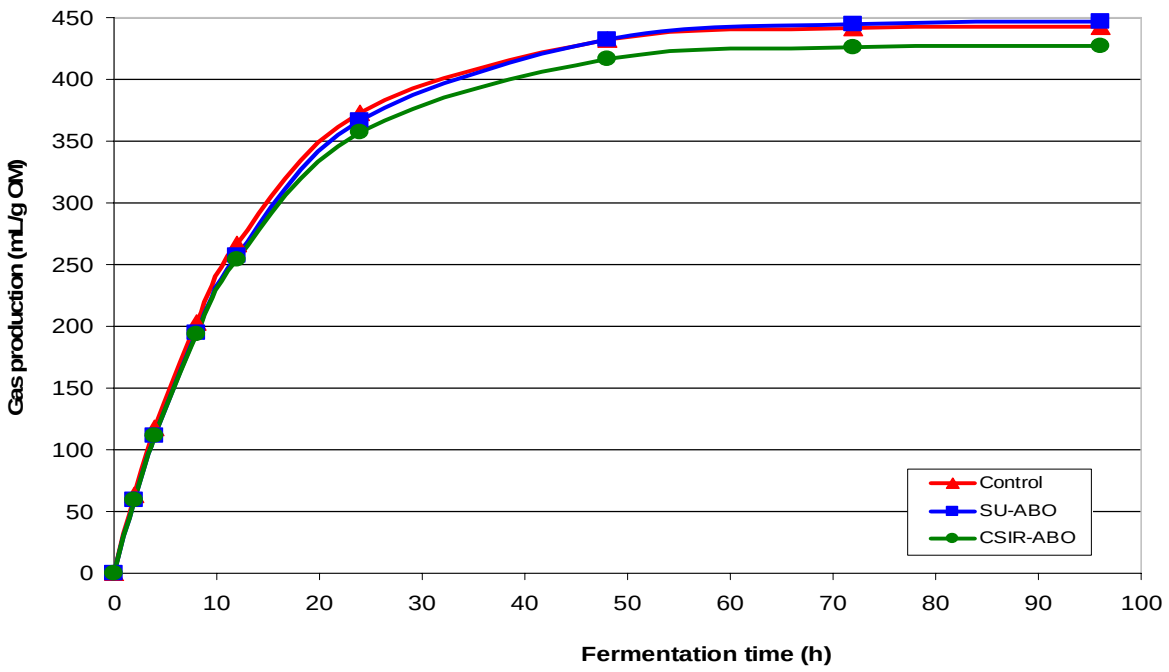

Figure 4.7 *In vitro* fermentation (ml/g OM) of a high concentrate diet (80%) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

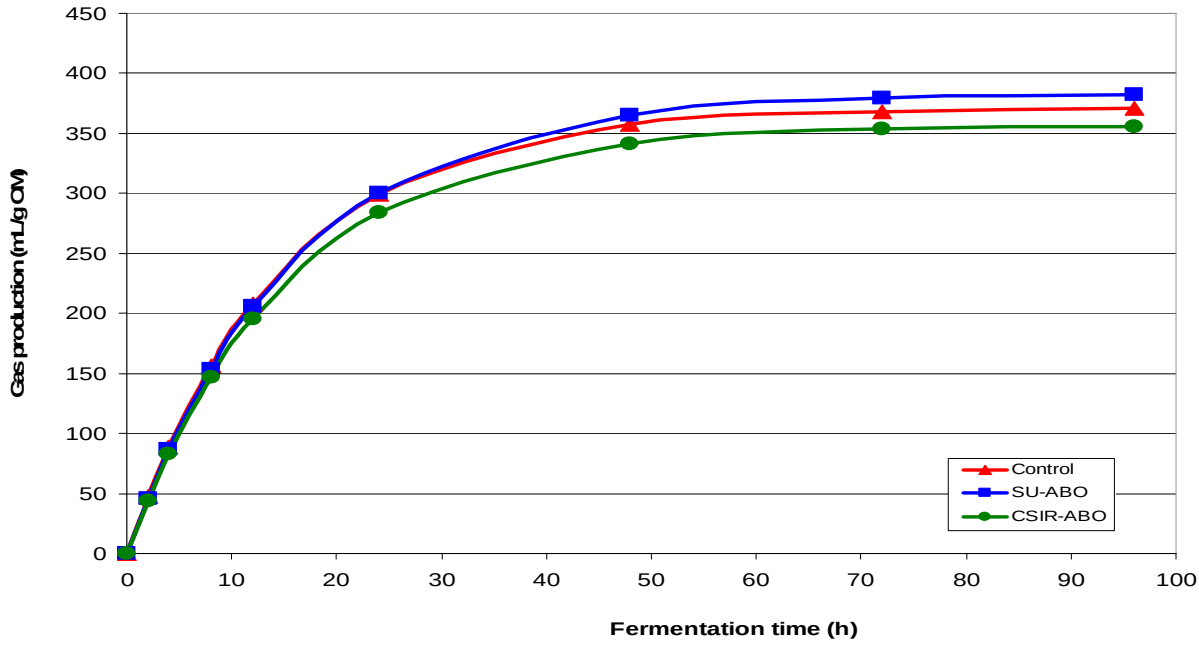

Figure 4.8 *In vitro* fermentation (ml/g OM) of a medium concentrate diet (50%) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes treatment (SU-ABO374, CSIR-ABO374) for 96 h.

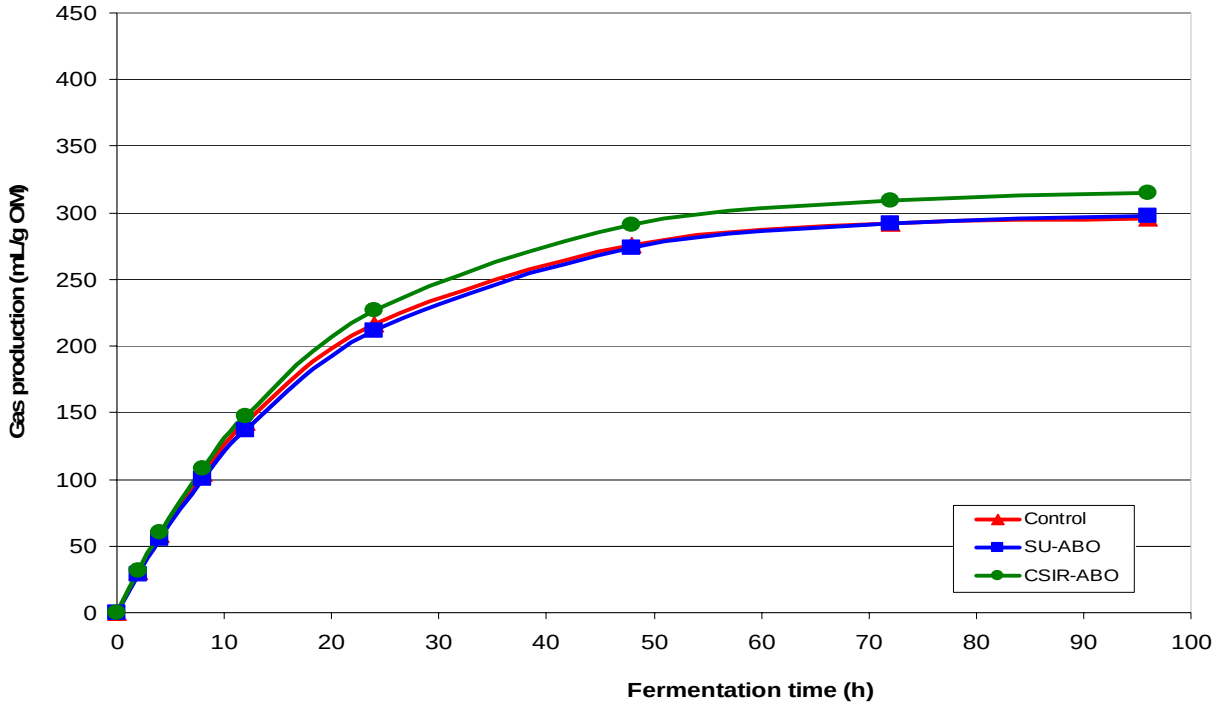

Figure 4.9 *In vitro* fermentation (ml/g OM) of a low concentrate diet (20%) incubated with buffered medium, rumen fluid and exogenous fibrolytic enzymes (SU-ABO374 and CSIR-ABO374) for 96 h.

Previous studies (Wang *et al*., 2001; Giraldo *et al.,* 2004) have shown that a pre-treatment of feed with exogenous enzymes before feeding or incubation with rumen fluid enhanced rumen fermentation. Kung *et al*. (2000) have suggested that this could be due to the stable enzyme-feed complex, and others have indicated the possibility of alteration in the fibre structure, which would stimulate microbial colonization (Nsereko *et al*., 2000; Giraldo *et al*., 2007a). In the present study, all enzymes did not have an effect on both DM and NDF degradation, and gas production for the diets. The study suggest that for lack of effects may have been influenced by factors such as pre-treatment effects (enough time not given for exogenous enzymes colonize so as to solubilze the fibre portion of the diet before adding rumen fluid for incubation), rumen temperatue and pH, also the mixed fibre portion of the concentrate diets not being a target substrate for the activities contained by the exogenous enzymes.

Results suggest that exogenous enzyme treatment effects on gas production for different concentrate diets were influenced by the proportion of forage: concentrate ratio and composition of the diet. Exogenous enzyme SU-ABO374 and CSIR-ABO374 resulted in higher gas production from HC than with LC, with variability in the interaction of enzymes with different substrate diets. Exogenous enzyme SU-ABO374 had indicated more gas produced from HC and MC, While LC had more gas produced from CSIR-ABO374. Beauchemin *et al*. (2004) stated that the lack of response to enzyme supplementation may be an indicating over or insufficient supply of enzymes.

Variability and lack of response from the exogenous enzymes may have been influenced by other factors such as diet type level of the enzyme activities provided, temperature and pH of the rumen environment for fibre digestion. Regarding the different proportion of the diets, may be the readily fermentable portion of the diet may have altered the pH and reduced the growth of the enzymes for fibre digestion. Therefore the study suggests that effect of the exogenous enzymes (SU-ABO374 and CSIR-ABO374) *in vitro* depend on the source, diet type and composition and the specific enzyme activities contained within the enzyme preparations.

4.7 CONCLUSION

Treatment of concentrate diets (80%, 50% and 20% concentrate) with the exogenous fibrolytic enzymes SU-ABO374 and CSIR-ABO374 did not appear to affect DM and NDF degradation as measured with the *in vitro* nylon bag technique. Enzyme treatments did not have an effect on the fermentation characteristics of diets as measured with the *in vitro* gas production method. When the residues of the gas production trials were taken as an estimate of degradation over 96 hours, treatments had different effects and the interactions that were observed complicated interpretation. No clear pattern could be observed. In the current trial, it was concluded that the enzymes SU-ABO374 and CSIR-ABO374 had no clear effect on DM or NDF degradation or fermentation characteristics of concentrate diets. High concentrate diets degraded at a higher rate than medium concentrate, which again degraded faster than low concentrate diets, regardless of enzyme treatment. Effective degradation was higher for high concentrate diet than for medium concentrate diet, which was higher than that of low concentrate diet.

Therefore, lack of response may have been due to factors such as pre- treatment effects, reduced stability and enzymes activities, optimum temperature and pH of the enzymes.

4.8 REFERENCES

Beauchemin, K.A. & Rode, L.M., 1997. Feed enzymes for ruminants. In: feature article. Feed Mix. 5(3), 1-2.

Beauchemin, K.A., Rode, L.M. & Karren, D., 1999. Use of feed enzymes in feedlot finishing diets. Can. J. Anim. Sci. 79, 243-246.

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 (Suppl. 2), E37–E47.

Beauchemin, K.A., Colombatto, D., Morgavi, D.P., Yang, W.Z. & Rode, L.M., 2004. Mode of action of exogenous cell wall degrading enzymes for ruminants. Can. J. Anim. Sci. 84, 13-22.

Giraldo, L.A., Ranilla, M.J., Tejido, M.L., Carro, M.D., 2004. Effect of enzyme application method on *in vitro* rumen fermentation of tropical forages. J. Anim. Feed Sci. 13, 63–66.

Giraldo, L.A, Ranilla, M.J., Tejido, M.L., Carro, M.D., 2007a. Influence of exogenous fibrolytic enzyme and fumarate on methane production, microbial growth and fermentation in Rusitec fermenters. Br. J. Nutr., doi:10.1017/S0007114507744446.

Giraldo, L.A., Tejido, M.L., Ranilla, M.J. & Carro, M.D., 2007b. In press: Effects of exogenous fibrolytic enzymes on *in vitro* rumen fermentation of substrates with different forage: concentrate ratios. Anim Feed Sci. Technol., doi:10-1016/j.animfeedscience.2007.06.013.

Goosen, L., 2004. The effect of an exogenous fibrolytic enzyme on forage digestibility parameters. M.Sc. (Agric) thesis, University of Stellenbosch, Stellenbosch, South Africa.

Hristov, A.N., Rode, L.M., Beauchemin, K.A. & Wuerfel, R.L., 1996. Effect of a commercial enzyme preparation on barley silage *in vitro* and *in sacco* dry matter degradability. In Proc. Western Section, Am. Soc. Anim. Sci. pp. 282-284.

Krause, M., Beauchemin, K.A., Rode, L.M., Far, B.I. & Nørgaard, P., 1998. Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Anim. Sci. 76, 2912-2920.

Kung, L.J., 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.

Mertens, D.R., 1997. Creating systems for meeting fiber requirements of dairy cows. J. Dairy Sci. 80, 1463-1481.

Nsereko, V.L., Morgavi, D.P., Beauchemin, K.A. & McAllister, T.A., 2000. Effects of fungal enzyme preparations on hydrolysis and subsequent degradation of alfafa hay fiber by mixed rumen microorganisms *in vitro*. Anim. Feed Sci. Technol. 88,153-170.

Ørskov, E.R. & McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighed according to rate of passage. J. Agric. Sci., Camb. 92, 499-503.

Piwonka, E.J. & Firkins, J.L., 1993. Effect of glucose on fiber digestion and particle-associated carboxymethylcellulase activity *in vitro*. J. Dairy Sci. 76,129-139.

Rymer, C., Huntington, J., Williams, B. & Givens, D., 2005. *In vitro* cumulative gas production techniques: History, methodological considerations and challenges. Anim. Feed Sci. Technol., 123-124, 9-30.

Selinger, L.B., Forsberg, C.W. & Cheng, K.J., 1996. The rumen: A unique source of enzymes for enhancing livestock production. Anaerobe (review) 2, 263-284.

Shriver, B.J., Hoover, W.H., Sargent J.P., Crawford, Jr. R.J., &. Thayne, W.V., 1986. Fermentation of a high concentrate diet as affected by rumen pH and digesta flow. J. Dairy Sci. 69, 413-419.

Statistica, 6.1. 2006. StatSoft, Inc., US

Titi, H.H. & Tabbaa, M.J., 2004. Efficacy of exogenous cellulose on digestibility in lambs and growth of dairy calves. Livestock Prod. Sci. 87, 207-214.

Vicini, J.L., Bateman, H.G., Bhat, M.K., Clark, J.H., Erdman, R.A., Phipps, R.H., Van Amburgh, M.E., Hartnell, G.F., Hintz, R.L. & Hard, D.L., 2003. Effect of feeding supplemental fibrolytic enzymes or soluble sugars with malic acid on milk production. J. Dairy Sci. 86, 576-585.

Wallace, R.J., Wallace, S.J.A.., McKain, N., Nsereko, V.L. & Hartnell, G.F., 2001. Influence of supplementary fibrolytic enzymes on the fermentation of corn and grass silages by mixed ruminal microorganisms *in vitro*. J. Anim. Sci. 79, 1905-1916.

Wang, Y., McAllister, T.A., Rode, L.M., Beauchemin, K.A., Morgavi, D.P., Nsereko, V.L., Iwaasa, A.D., Yang, W., 2001. Effects of an exogenous enzyme preparation on microbial protein synthesis, enzyme activity and attachment to feed in the rumen simulation technique (Rusitec). Br. J. Nutr. 85, 325–332.

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.

Yang, W.Z., Beauchemin, K.A. & Rode, L.M., 2000. A comparison of methods of adding fibrolytic enzymes to lactating dairy diets. J. Dairy. Sci. 11, 1-9.

GENERAL CONCLUSION

Forages are an important source of nutrients in ruminant production as they serve as the major source of fibre constituencies such as CF, ADF, NDF, cellulose, hemicellulose and lignin. However, forage utilization by ruminant animals is limited by the availability of low quality (high NDF content and low nutritive value) and lack of constant supply of grasses and legumes. Exogenous fibrolytic enzymes have been defined to have the potential to improve the degradability of mature forages and concentrate based diets, thereby enhancing the digestibility of structural carbohydrates by rumen microbes.

Results from *in vitro* DM and NDF degradation and gas production of mature forages and concentrate diet substrates indicated that the exogenous application of the fibrolytic enzyme cocktails SU-ABO374 and CSIR-ABO374 did not differ on DM and NDF degradation and gas production of forage and concentrate diet substrates. From the results reported in this study, different forages and concentrate diets differed in terms of NDF degradation and gas production, but exogenous enzyme treatment had no effect.

It appears that the activities of exogenous fibrolytic enzyme extracts were not sufficient to work synergistically with rumen microbes as neither the rate or DM and NDF degradation and gas production for forages or concentrate diets was enhanced. However, lack of response may have been influenced by pre-treatment effects, reduced stability and enzymes activities. Other factors such as optimum temperature of the exogenous enzymes may also have influenced no effect results, due to lacking of enough information on the activities of the enzymes, such as specific enzymes contained in the cocktail, optimum temperature and suitable pH due to confidentiality from the manufactures. It is not clear whether the effects of the exogenous enzymes were influenced by the environment (rumen pH and temperature) or other factors mentioned above.

Furthermore, the usage of exogenous enzymes in ruminant diets is limited by variability of responses reported from literature. Trial results are sometimes published without information regarding the enzyme type, concentration and activity, substrate type activity, or with enzyme activities measured at temperatures and pH that differ from conditions in the rumen. Information is therefore not always sufficient to support current results. Rumen conditions may also cause a loss of

fibrolytic enzyme activity, such that no responses in feed intake, digestibility and production will be observed following enzyme application.

Results of the current trials suggest that the exogenous fibrolytic enzymes SU-ABO374 and CSIR-ABO374, when added directly to the incubation medium and without a substantial pre-incubation interaction time, were ineffective to improve DM and NDF degradation or gas production of mature forages and concentrate-forage diets *in vitro*. Further research is required on refinement of the cocktails of the enzyme supernatants SU-ABO374 and CSIR-ABO374 for application to mature forages and concentrate based diets. More information on the activities of these enzymes is needed for improved application on ruminant diets.