Improving Forage Quality and Animal Performance with Fibrolytic Enzymes

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Introduction

Enzymes have been used for decades to improve the utilization of swine and poultry diets. For instance phytase, amylase, ß-glucanase and xylanase are added to the cereal-based diets of such monogastrics to increase the utilization of dietary phosphorous, starch, ß-glucans and arabinoxylans respectively. For many years, researchers were discouraged from using enzymes to enhance the utilization of ruminant diets because of perceptions that the hydrolytic capacity of the rumen could not be enhanced by supplemental enzymes, and concerns that such enzymes would be ineffective due to ruminal proteolysis. These concerns have been disproved by several recent studies that have demonstrated that fibrolytic enzyme supplementation enhances the productivity of livestock, and several fibrolytic enzyme products are currently commercially available. However in vitro and animal-based studies on the effects of fibrolytic enzyme application to feeds have not been unanimously supportive of their benefits. The interplay of several enzyme, host, feed and management – related factors determine the effectiveness of enzymes in hydrolyzing feed components. Several excellent reviews have been published on this subject (Kung 2001a, b; McAllister et al., 2001; Beauchemin et al., 2003; Beauchemin et al., 2004). However, these have either only focused on direct fed enzyme application with the exclusion of enzyme application at ensiling, and have not considered the potential of using enzymes to improve the quality of tropical forages. The intention in this paper is to review the literature on the effects of enzyme application to feeds at ensiling or feeding, and the determinants of such effects, and to present the results of certain preliminary studies aimed at improving the utilization of tropical forages with fibrolytic enzymes.

Enzyme application at ensiling

Enzyme application at ensiling is practically attractive because uniform distribution throughout the forage is ensured when enzymes are applied using properly calibrated sprayers on forage harvesters. There is also a sound theoretical basis for applying fibrolytic enzymes to forages at ensiling. If effective, such enzymes should hydrolyze plant cell walls into simple sugars that can be used as fermentable substrates by homolactic bacteria. Therefore fibrolytic enzyme application should make the silage fermentation more homolactic and result in a reduction in proteolysis and dry matter (DM) losses in addition to increasing the digestibility of the forage. Several silage additives contain a mixture of inoculant bacteria and fibrolytic enzymes in order to ensure that sufficient homofermentative bacteria are available to utilize the sugars

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released by enzyme action and dominate the fermentation. Table 1 summarizes published reports on how enzyme application at ensiling affects silage fiber concentration, and demonstrates that in most cases fiber concentration is reduced by enzyme treatment. Several studies have also demonstrated that enzyme application especially in the presence of microbial inoculants improves the fermentation of tropical grasses (Rodriguez et al., 2001; Adesogan et al., 2004), cool season grasses (Selmerolsen et al., 1993; Smith et al., 1993; Beuvink and Spoelstra, 1994; Ridla and Uchida, 1999; Rodrigues et al., 2001b), alfalfa (Selmerolsen et al., 1993; Smith et al., 1993; Beuvink and Spoelstra, 1994; Ridla and Uchida, 1999; Rodrigues et al., 2001b) and wheat silage (Tengerdy et al., 1991; Hristov, 1993; Hoffman et al., 1995; Sheperd et al., 1995; Nadeau et al., 2000), (Froetschel et al., 1991; Adogla-Bessa and Owen, 1995; Adogla-Bessa et al., 1999). Although some studies have also shown that enzyme application improves the fermentation of corn silage (Colombatto et al., 2004), many others have not (Chen et al., 1994; Stokes and Chen, 1994; Sheperd and Kung, 1996a, b). This discrepancy may be due to varietal differences between the hybrids used in the studies. In general terms, the high sugar content of corn (and sorghum) implies that fermentable substrate availability usually does not limit the fermentation of these forages. Enzyme treatment of such forages can stimulate yeast proliferation due to excessive sugar concentrations. Selmerolsen (1994) also showed that the fermentation of crops with low sugar contents was improved more by enzyme addition, while that of crops with high sugar contents was improved more by lactic acid bacteria inoculation. In agreement we have also shown that treatment of bermudagrass, which is low in sugars with fibrolytic enzymes alone (Dean et al., 2005) or with an enzyme-inoculant blend (Adesogan et al., 2004) improved the fermentation, but contradictory results exist (Mandevbu et al., 1999). Clearly enzyme application at ensiling to forages containing low sugar contents is logical, but the response depends on the enzyme activities and treatment conditions. Kung (2001b) showed that over a five-year period, enzyme treatment at ensiling had increased feed intake, gain and milk production in 28, 40 and 33 % of studies respectively. Although few, if any studies have compared the relative merits of applying enzymes at feeding or ‘at ensiling’, most of the recent interest in the subject has focused on the former.

Enzyme application at feeding

Enzyme application to diets at feeding is attractive because the fermentable substrates released by enzyme action can be directly fermented by ruminal bacteria, thereby releasing energy for the host animal. However care is needed to ensure an even distribution of the small quantity of enzyme that is typically added. Nevertheless, several studies have demonstrated that enzyme application at feeding improves milk production in dairy cows and improves average daily gain in beef cattle (Table 2).

Mode of enzyme action

Direct-fed enzyme application is often accompanied by increased feed intake, which is attributable to increased palatability due to sugars released by pre-ingestive fiber hydrolysis, post-ingestive enzyme effects such as an increased digestion rate or extent of digestion or increased passage rate. These factors reflect an increase in the

hydrolytic capacity of the rumen which indirectly reduces gut fill, and hence enhances feed intake.

In other studies, enzyme treatment has increased animal performance without increasing feed intake. This may be due to reduced digesta viscosity (Hristov et al., 2000) and alterations in ruminal fermentation. Dawson and Tricarico (1999) showed that when fescue hay was not treated or treated with preparations high in either xylanase or cellulase activity, xylanase addition increased carbohydrate utilization and VFA production, cellulase addition altered VFA proportions, and addition of a combination of the enzymes increased carbohydrate digestion and increased the acetate:propionate ratio. In addition, exogenous enzymes have also been shown to stimulate fibrolytic bacteria and increase their attachment to fiber particles. Newbold (1997) conducted a series of experiments aimed at determining how crude fungal extracts enhanced the numbers of ruminal cellulolytic bacteria. They implicated a high molecular weight fraction in the extract that was destroyed by proteases and had similar fibrolytic activity to cellulase, and concluded that polysaccharidase enzymes were the most likely cause of the increased bacterial numbers. Newbold also showed that crude fungal extract from *Aspergillus foetidus* can stimulate the attachment of rumen microbes to plant fibers in vitro and in vivo. They speculated that this was due to chemotactic responses to soluble sugars released from plant fibers by the polysaccharidase enzymes, which initiated the attachment of fungi and protozoa to plant cell walls in the rumen. This was considered to be how a small quantity of fibrolytic product exerts a significant effect on fiber degradation in vitro. Synergistic action between *Trichoderma longibrachiatum* fibrolytic enzymes and mixed ruminal microorganisms also increased the hydrolysis of soluble cellulose, xylan and corn silage by 35, 100 and 40% (Morgavi et al., 2000).

Factors affecting enzyme action

It is evident from Table 2, that there is a wide range in responses to supplementation with direct fed-enzymes. Some of the reasons for the variation are given below:

Enzyme product composition

Cellulase and xylanase are generic terms for groups of specific enzyme activities, such that two products with identical labels for enzyme level may differ in effects on ruminal fiber digestion, and failure (or success) of one product does not guarantee that of a seemingly identical product (Siciliano-Jones, 1999). For instance ‘cellulase’ enzymes are a complex of various endo-and exo-betaglucanases, cellobiohydrolase and cellobiase (Hristov et al., 1998), yet cellulase is often thought to be a single enzyme. Furthermore, several papers on animal responses to enzyme supplements are published without reference to enzyme activity, or with enzyme activities measured at temperatures and pH that differ from that in the rumen, such that the potential activity of such products is overestimated. Ruminal conditions can cause a loss of fibrolytic enzyme activity, such that no responses in feed intake and milk production will be seen following enzyme application (Vicini et al., 2003).

Mode and time of enzyme delivery

Previous calls for more research on pre-feeding storage times of enzyme-treated dietary components (Wallace et al., 2001), led to in vitro and in vivo studies in which enzymes were added immediately or 24 h prior to feeding. However since such studies showed no differences due to time of enzyme treatment it has been suggested that there is little or no requirement for a reaction phase for enzymes added to diets (Beauchemin et al., 2003). However, more research is required in this area since many studies now involve enzyme addition to concentrates at milling and entail enzyme-diet interaction periods of up to one month. Depending on storage conditions, enzyme activity may be reduced by such protracted periods.

Intraruminal dosing of exogenous enzymes did not affect apparent digestibility of DM, crude protein (CP) or neutral detergent fiber (NDF) but reduced rumen pH and the activity of key endogenous fibrolytic enzymes and also increased the soluble DM fraction and effective DM degradability (Hristov et al., 2000). Earlier work by these authors (Hristov et al., 1998) showed that abomasal infusion or dietary supplementation with exogenous enzymes did not increase DM intake, in situ degradation or total tract digestion in cattle. No differences were also found between dietary concentrate or TMR supplementation or rumen infusion with enzymes on DM intake digestibility or milk yield in dairy cows (Sutton et al., 2003). These studies suggest that post-ingestive supply of fibrolytic enzymes is no more effective than dietary supplementation for increasing feed intake, digestion and milk yield in cattle. It is not clear why dietary treatment was not effective in the studies above, since this mode of delivery is the key to harnessing the potential of exogenous enzymes in ruminant nutrition (Wallace et al., 2001).

Ruminal activity and stability of direct-fed enzymes

Enzyme activity is dictated by several factors including presence of inhibitors and co-factors, prevailing pH, moisture, temperature and concentration of enzyme and substrate. A common error is the determination of enzyme activity under conditions that optimize enzyme action but differ considerably from the ruminal environment, such that measured enzyme activity is overestimated. Clearly, if the enzyme is expected to exert most of it's' effect in the rumen, the enzyme activity should be measured under conditions that mimic the ruminal environment. Adoption of recently proposed methods for standardizing fibrolytic enzyme activity measurement (Colombatto and Beauchemin, 2003) should help in this regard.

Dawson and Tricarico (1999) suggested that the most active period for enzyme effects is in the first 6 – 12 h of the digestive process, though they also speculated that such action occurs prior to bacterial colonization of feed substrates or action of endogenous enzymes. In support, Newbold (1997) noted that enzymes must function within a few hours of feeding before being degraded by the proteolytic activity of rumen microbes. The likelihood of ruminal proteolysis limited the use of enzymes in ruminant feeds for decades. However, Morgavi et al., (2001) found that four commercial enzymes were stable when incubated in rumen fluid, pepsin or pancreatin, and adduced this to carriers and stabilizers, manufacturing processes and enzyme-substrate interactions.

Host proteases and the acid pH of the abomasum are more likely to degrade exogenous enzymes than ruminal proteases (Hristov et al., 1998; Morgavi et al., 2001). Sustained enzyme stability in the rumen can result from natural or artificially induced enzyme glycolysation, which involves covalent bonding of monosacharides to specific amino acid side chains in enzymes (van de Vyver et al., 2004). Glycolysation has been shown to confer resistance to proteolysis in monogastrics and ruminal fluid (van de Vyver et al., 2004), but non glycosylated enzymes may also resist ruminal proteolysis due to adaptation over time and their genetic composition (Fontes et al., 1995). However several different enzyme preparations are commercially available, and lack of response to enzyme treatment in some of the studies may be attributed to ruminal enzyme instability. For instance (Vicini et al., 2003) attributed the lack of response to enzyme treatment in their study to higher ruminal pH and lower ruminal temperature than the optima for the fibrolytic activities in their enzyme preparation. Therefore there are notable variations in the stability of commercially-available enzyme preparations and their rumen stability should be verified before they are used in practice.

Enzyme- feed specificity and the portion of the diet to which enzymes are applied

The following studies reveal the importance of matching enzymes to specific substrates: Beauchemin et al. (1997) reported greater responses when enzymes were applied to dry forages instead of wet forages. Feng et al. (1996) showed that direct-fed enzymes were more effective when applied to dried grass at feeding than to freshly cut, dried grass at harvest or wilted dried grass after harvest. When the same enzyme was applied to hay and corn silage, it increased the NDF digestion of corn silage but not hay (Siciliano-Jones, 1999). Also application of the same enzyme to alfalfa and ryegrass increased the digestibility of alfalfa but not ryegrass (Pinos-Rodriguez et al., 2002). Further evidence for enzyme-feed specificity is apparent from studies in which enzymes were added a specific dietary component. Bowman et al. (2002) found that enzyme application to the concentrate (45% of total mixed ration, TMR) instead of a pelleted supplement (4 % of TMR) or a premix (0.4% of TMR) did not affect intake, salivation or rumen function but numerically increased fat-corrected milk yield compared to control cows. They therefore concluded that the proportion of the diet to which the enzyme is applied must be maximized to ensure a beneficial response. In contrast, (Yang et al., 2000) showed that applying enzymes to the concentrate was more effective than applying them to the TMR in terms of the response in milk yield and digestibility of DM, organic matter (OM) and CP. However other studies found no differences in milk yield and intake when enzymes were applied to TMR or forage (Vicini et al., 2003) or to TMR or concentrate (Phipps et al., 2000; Sutton et al., 2003) or to alfalfa cubes and the concentrate (Yang et al., 1999). Since concentrates are ruminally readily fermented and contain low fiber concentrations, the beneficial effects of enzyme addition to this dietary fraction may be due more to synergistic effects on microbial populations and endogenous enzyme secretion, than to direct cell wall hydrolysis. Also, the study in which enzyme application to concentrate proved more effective (Yang et al., 2000) had a lower forage to concentrate ratio (38:62) than those (57:43, 57:43, 55:45, and 60:40) in which it did not (Yang et al., 1999; Phipps et al., 2000; Sutton et al., 2003; Vicini et al., 2003). Therefore the effect of the dietary component to which the enzyme is added

may depend on the forage to concentrate ratio and the uniformity of enzyme application to that component.

Level of enzyme application

Several studies have shown that applications of high levels of enzymes to forages or diets produce less desirable responses than low levels. For instance Lewis et al. (1999) noted that a medium level of enzyme supplementation produced more milk than a low or high level of application, and Beuchemin et al. (2000) found that a high level of enzyme application was less effective than a low level at increasing total tract digestibility. The reason for the poor response to the low enzyme level is obvious, but that for the higher level is less apparent. It may be partly attributed to negative feedback inhibition which is one of the classical modes of regulation of enzyme action. This feedback mechanism occurs when enzyme action is inhibited by production of a critical concentration of a product of the enzyme-substrate interaction. For instance fermentation of sugars produced by cell wall hydrolysis may reduce ruminal pH to levels that inhibit cell wall digestion. An alternative hypothesis is that excessive enzyme application blocks binding sites for enzymes or may prevent substrate colonization (Beauchemin et al., 2000; Beauchemin et al., 2003). The fact that enzymes can be overfed or underfed makes their application complex (Dawson and Tricarico, 1999) and underscores the need for determining the optimal level of application for each enzyme preparation. A more disconcerting observation is that in vitro evaluation of the activities of two fibrolytic enzymes revealed that when added at the rates recommended by their manufacturers, the enzymes would not increase significantly glycanase and polysaccharidase activities in rumen fluid unless much higher application rates are used (Wallace et al., 2001). This highlights the need for further in vivo studies to verify the application rates and activities of some commercially available enzymes.

Stage of lactation of dairy cows

Theoretically direct-fed enzyme supplementation should be most effective when ruminal fiber digestion is compromised due to factors like acidosis, or when dietary glucose supply is inadequate to meet the needs of the cow such as in early lactation. In support, direct-fed enzyme supplementation has increased milk production from cows in early lactation, but not from cows in mid lactation (Schingoethe et al., 1999), and has increased weight gain, milk production and feed intake in early lactation, but not in late lactation (Knowlton et al., 2002). Also when cows in positive energy balance were fed enzyme supplemented diets, increased intake of digestible energy due to enzyme supplementation did not increase milk yield (Beauchemin et al., 2000). In contrast, Lewis et al. (1999) showed that enzyme supplementation increased milk yield in early or mid lactation in two separate experiments. Also Zheng et al. (2000)(2000) found that stage of lactation did not affect the increase in milk production due to enzyme-supplementation, but concluded that delaying enzyme supplementation till 6 weeks postpartum resulted in a loss of 280 kg of milk in the first 18 wk of lactation, and therefore recommended starting to feed enzyme-supplemented diets soon after parturition. The discrepancies between the studies cited above are due to factors such as differences in dietary components, forage to concentrate ratio and enzyme

composition and activity. More studies are needed to conclusively demonstrate the optimal stage of lactation for feeding fibrolytic enzymes to dairy cows.

Recent studies with fibrolytic enzymes at the University of Florida

Most of the studies involving the use of fibrolytic enzymes for enhancing ruminant feeds have focussed on enhancing the quality and utilization of cool-season forages. Yet warm-season, C4 grasses tend to have greater concentrations of fiber as well as lignin and phenolic acids that impede digestion (Borneman et al., 1990; Jung and Allen, 1995; Krueger et al., 2003). Therefore, there is greater scope for digestibility enhancement with enzymes in C4 grasses, if enzymes that can effectively hydrolyze the recalcitrant fibrous components of such grasses are used. The following recent studies at the University of Florida have been based on this premise and they focus on an area in which information is limited.

Effect of fibrolytic enzymes on the fermentation and quality of bermudagrass silage

Dean et al. (2005) compared the efficacy of four proprietary cellulase, xylanase preparations for improving the digestion and fermentation of five-week regrowth, Tifton 85 bermudagrass silage. They found that although all the enzymes had some beneficial effects on the fermentation, one enzyme (Promote, Cargill Corp., St. Louis, MO) proved to be outstanding. This enzyme hydrolyzed the cell walls in the grasses into sugars, which stimulated the growth of homolactic bacteria and resulted in reductions in DM losses, pH, proteolysis and water-soluble carbohydrate utilization, and an increase the lactic acid concentration (Table 3). This enzyme also increased the 6 and 48 h DM digestibility and the 48 h NDF and acid detergent fiber (ADF) digestibility of the grass. This study clearly demonstrated that preparations that contain similar enzymes can have different effects on polysaccharide hydrolysis, because of differences in the concentrations and activities of the component enzymes. A follow up study is currently investigating the effect of applying this enzyme to different components of a bermudagrass silage-based TMR on feed intake, digestion and milk production in dairy cattle. Some of the results of this study will be presented at the Symposium.

Effect of fibrolytic enzymes or ammonia on the nutritive value of tropical grass hays

The effect of applying ammonia or different rates of the fibrolytic enzymes used in the previous study on the nutritive value of twelve-week regrowths of Coastal bermudagrass and Pensacola bahiagrass was studied in two experiments. In both experiments, DM digestibility after 6 hours was affected by forage type and bermudagrass was consistently less digestible than bahiagrass (Tables 4 and 5). In the first experiment, the enzyme which proved to be most effective for bermudagrass silage in the above study (Promote) did not affect the CP concentration or DM digestibility of the hays, but did increase their NDF digestibility. Whereas ammonia application increased ($P<0.05$) all of these variables. In the second experiment, treatment with ammonia, or the enzymes increased ($P<0.05$) the 6 h DM digestibility, but only one

enzyme (Biocellulase X20, Lodestar, IL) and ammonia treatment increased the 48 h DM digestibility (Table 5). This enzyme was more effective than the other enzymes at increasing 6 h DM digestibility. Ammonia treatment was more effective ($P<0.05$) than the enzyme treatments at increasing 6 or 48 h DM digestibility. Only ammonia treatment increased ($P<0.05$) CP concentration and 6 and 48 h NDF digestibility, though another enzyme (Biocellulase A20, Lodestar, IL) also tended ($P = 0.059$) to increase 6 h NDF digestibility.

These results indicate that treatment with ammonia or enzymes enhanced the initial phase of the digestion of the hays but only X20 and ammonia treatment enhanced the latter phase of digestion. This study therefore contradicts reports that fibrolytic enzymes do not increase the extent of forage digestion. However the enzymes were not as effective as ammonia at increasing CP and DM or NDF digestibility in the hays, suggesting that more appropriate enzyme mixtures need to be used for enhancing the nutritive value of mature, C4 grasses.

Effect of esterase enzymes on the fermentation of tropical grass hays

A third aspect of the fibrolytic enzyme studies at the University of Florida is based on the premise that the activities of cellulase and hemicellulase enzymes alone are insufficient for effectively hydrolyzing cell walls in C4 grasses due to the presence of arabinoxylan ferulate ester and ether linkages which impede digestion (Jacobs and McAllan, 1991). Though such etherified cross linkages are not known to be degraded by anaerobic microorganisms, esterified cross linkages can be degraded by ferulic acid esterase enzymes (Choung and Chamberlain, 1992). Inclusion of fungal phenolic acid esterases in the enzyme mixture has increased polysaccharide hydrolysis from bermudagrass cell walls over that of polysaccharidases alone (Rodrigues et al., 2001a). Recent studies at the University of Florida have also shown that enzyme preparations containing high ferulic acid esterase activity as well as xylanase and cellulase activity reduced the NDF and ADF concentrations and increased the digestion of hays made from twelve-week regrowths of Tifton 85 bermudagrass, Coastal bermudagrass and Pensacola bahiagrass (Table 6) (Krueger et al., 2003). The enzyme also increased rate and extent of *in situ* degradation of the forages and reduced the lag time before forage degradation commenced (Krueger et al., 2004). These studies suggest that enzyme treatment can improve the nutritive value of tropical forages. Future studies will determine the effect of different ratios of esterase enzymes to polysaccharidases on their nutritive value.

Conclusions

Important progress has been made in using fibrolytic enzymes to enhance silage conservation and to increase meat and milk production from livestock. However more research is needed in the areas of determining the most critical activities for inclusion in commercial preparations, improving enzyme-feed specificity and developing practical guidelines that ensure the effectiveness of the enzymes. Studies on fibrolytic enzyme application to tropical silages have been promising, but indicate that commercial products containing similar enzymes vary in their effects. Studies on fibrolytic enzyme

application to tropical hays have shown some benefits, but ammonia application is more effective. The current challenge is to develop appropriate combinations of enzymes that are as effective at ammonia at hydrolyzing tropical grasses.

Table 1. Effect of enzyme application at ensiling on neutral (NDF) and acid (ADF) detergent fiber concentration (g/kg DM) of forages.

	Change in fiber concentration	
Source	NDF	ADF
Grasses		
Beuvink and Spoelstra (1994)	-35.6	-
Jacobs and McAllan (1991)	-4.9	-10.2
Choung and Chamberlain (1992)	-12.3	-11.7
Mandebvu et al. (1999)	0	0
Rodrigues et al. (1993)	-29.6	-19.2
Selmer-Olsen et al. (1993)	-26.9	-30.7
Stokes et al. (1996)	-5.3	-8.2
Weinberg et al. (1993)	0	-8.8
Legumes and grass-legume silages		
Kung et al. (1991)	+1.1	+2.6
Fredeen and Mc Queen (1993)	-1.3, -7.8	-1.4, -6.5
Hoffman et al. (1995)	-6.7	-2.0
Sheperd et al. (1995)	-9.5, -7.8	-9.2, -4.2
Nadeau and Buxton (1997)	-3.7	0
Whole plant silages		
Kung et al. (1990)	-4.2	-2.8
Weinberg et al. (1993)	-7.3	-7.2
Adogla-Bessa et al. (1999)	-8.5	-12.8
Adogla-Bessa et al. (1999) (+ urea)	+0.3	-1.1
Nia and Wittenberg (1999)	-0.4	-0.6

Adapted from Colombatto and Adesogan (2005).

Table 2. Effect of enzyme treatment of feeds on the performance of dairy and beef cattle in some published studies.

Source	Change in milk production in dairy cattle (kg/d)[1]
Beauchemin et al. (1999b)	+0.30, +1.50
Lewis et al. (1999)	+1.20, +6.29, +1.60
Rode et al. (1999)	+3.59
Schingoethe et al. (1999)	+1.20, +0.90, +2.70, +1.30
Yang et al.	+0.90, +1.90, +1.60
Beauchemin et al. (2000)	-0.50, -0.50
Kung et al.(2000)	+2.50, -0.80, +0.70, +2.50
Yang et al. (2000)	-0.10, +2.10
Zheng et al. (2000)	+2.00, +4.09, +1.50

	Change in performance of beef cattle	
	Gain[1]	Feed efficiency[1]
Beauchemin et al. (1997)	+0.09	-0.77
Beauchemin et al. (1999a)	+0.13	-0.77
Zinn and Salinas (1999)	+0.08	-0.09
McAllister et al. (1999)	-0.04, +0.04, +0.08	+0.17, -0.16, +0.06
ZoBell et al. (2000)	-0.003, +0.001	-0.26, -0.74

[1] When more than one number is listed, several enzyme treatments were used. (Adapted from Kung (2001b) and (Colombatto and Adesogan, 2005)).

Table 3. Effect of fibrolytic enzyme treatment on the fermentation characteristics and chemical composition (g/kg DM) of bermudagrass silage (Dean et al., 2005).

Treatment[1]	pH	DM losses	NH_3N	NDF	Sugars	Lactic acid	NDF digestibility
Control	4.40	8.6	32	753	50	402	431
Pr	4.03	4.2	25	725	12.2	66	450
X-20	4.40	7.0	35	743	6.0	48	383
CT	4.40	7.3	30	743	6.9	56	416
A-20	4.33	6.5	33	753	5.3	56	429
S.E.	0.09	1.12	0.03	3.36	0.81	10.61	13.102
Contrasts				*P* values			
Control vs Pr	0.002	0.002	< 0.01	< 0.001	< 0.001	0.211	0.004
Control vs X-20	0.975	0.218	0.126	0.016	0.109	0.899	0.215
Control vs CT	0.992	0.316	0.248	0.015	0.014	0.635	0.371
Control vs A-20	0.516	0.120	0.630	0.977	0.407	0.612	0.085

[1] Cellulase-hemicellulase enzyme preparations: Pr, Promote; X-20, Biocellulase X-20; CT cellulase enzyme; A-20, Biocellulase A-20.

Table 4. Effect of treatment with Promote enzyme or ammonia on dry matter (DM) digestibility and crude protein concentration of tropical grass hays (Dean et al., 2003)

Treatment	Level	DM digestibility (g/kg) after				Crude protein	
		6h		48h		(g/kg DM)	
		Bermuda	Bahia	Bermuda	Bahia	Bermuda	Bahia
Control		93	142	486	488	66	67
Promote	0.5x	94	137	494	488	67	67
Promote	1x	100	142	475	478	64	68
Promote	2x	100	140	496	475	64	70
Ammonia		156	187	609	620	170	128
				P			
Level effect		ns	ns	ns	ns	ns	ns
Forage effect		<0.001		ns		ns	
Contrasts							
Control vs Promote		0.006	0.419	0.686	0.633	0.946	0.285
Control vs Ammonia		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Promote vs Ammonia		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Bermuda=bermudagrass; Bahia=bahiagrass.

Table 5. Effect of treatment with ammonia or fibrolytic enzymes on dry matter (DM) digestibility and crude protein concentration of hays (Dean et al., 2003)

Treatment	Level	DM Digestibility (g/kg) after 6 h		DM Digestibility (g/kg) after 48 h		CP (g/kg DM)	
		Bermuda	Bahia	Bermuda	Bahia	Bermuda	Bahia
Control		77	127	436	445	65	70
X-20	0.5x	106	142	497	476	69	72
X-20	1x	114	130	511	456	67	71
X-20	2x	113	129	514	474	65	70
X-20	Mean	111	134	506	469	67	71
X-20	Level effect	ns		ns		ns	
CT	0.5x	90	121	465	423	67	72
CT	1x	99	127	480	420	70	69
CT	2x	102	130	477	457	65	77
CT	Mean	97	126	474	433	67	73
CT	Level effect	ns		ns		ns	
A-20	0.5x	102	123	472	439	68	72
A-20	1x	116	120	482	467	68	70
A-20	2x	94	122	469	425	69	76
A-20	Mean	104	122	474	444	68	73
A-20	Level effect	ns		ns		ns	
Ammonia		137	181	569	599	163	130
Contrasts		*P*					
Forage effect		0.001		0.001		0.584	
Control vs X-20		0.003	0.001	0.002	0.064	0.086	0.713
Control vs CT		0.029	0.076	0.307	0.366	0.854	0.254
Control vs A-20		0.003	0.071	0.138	0.939	0.240	0.259
Control vs Ammonia		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
X-20 vs CT		0.031	0.027	0.002	0.002	0.009	0.271
X-20 vs A-20		0.267	0.026	0.001	0.007	0.001	0.281
CT vs A-20		0.266	0.985	0.524	0.215	0.167	0.991
Ammonia vs X-20		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ammonia vs CT		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Ammonia vs A-20		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1] Cellulase-hemicellulase enzyme preparations: X-20, Biocellulase X-20; CT cellulase enzyme; A-20, Biocellulase A-20.

Table 6. Effect of esterase enzyme application on 6 or 48 h in vitro dry matter digestibility (IVDMD), and 96 h in vitro rumen fluid-pepsin organic matter digestibility (IVOMD, Tilley and Terry, 1963) of tropical hays (Krueger et al., 2003).

	Enzyme application rate					Mean	S.E.M.	P^2	Forage contrasts		Forage*Enzyme	
Item[1]	0x	0.5x	1x	2x	3x				Bah v. Berm	C-B v. T-B	Bah v. Berm	C-B v. T-B
		6 hr. IVDMD										
BAH	133	159	165	156	182	159	9.87	NS				
C-B	180	185	178	207	212	192	6.52	0.02 Q				
T-B	99	113	97	149	159	123	8.81	0.001 L				
Mean	137	152	147	171	184		8.52	0.001 L	NS	0.001	NS	0.001 L
		24hr. IVDMD										
BAH	403	394	417	368	404	398	13.8	0.007 L				
C-B	398	380	399	396	392	492	13.8	NS				
T-B	355	389	358	398	388	377	16.8	NS				
Mean	386	387	391	387	395		14.9	0.03 L	NS	NS	0.03 Qt	NS
		96 hr. IVOMD										
BAH	398	396	406	424	405	405	5.1	0.005 C				
C-B	443	396	442	473	467	444	6.4	0.001 C				
T-B	437	435	450	461	449	447	4.3	0.003 Qt				
Mean	426	409	433	453	440		5.2	0.001 C	0.001	NS	0.05 Q	0.003 C

[1] BAH= bermudagrass, C-B = Coastal bermudagrass, T-B = Tifton 85 bermudagrass.
[2] L = linear, Q = quadratic, C = Cubic, Qt = quadratic.

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