Chemical Composition and Digestion Kinetics of Oat Silage and Urea Treated Wheat Straw as Influenced by Exogenous Fibrolytic Enzymes

By

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Abd Ur Rehman

DEDICATED TO

MY LOVING GRANDFATHER

Chaudhary Fazal Kareem

I would love him a lot, but Allah loved him more

Whose optimistic and positive living attitude will always be a source of inspiration for me

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Contents

List of tables

Abstract

Two independent experiments were conducted to evaluate the chemical composition and digestion kinetics of oat silage and urea treated wheat straw (WS) as influenced by varying level of exogenous fibrolytic enzymes. In experiment-I, fifty day old oat grass was ensiled with 2% molasses and 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme /Kg of dry matter (DM). Oat grass was ensiled in 36 laboratory silos under Completely Randomized Design for twenty one days. Chemical composition revealed that the DM and organic matter (OM) contents remained unaltered ($P>0.05$) across all treatments. Crude protein (CP), true protein (TP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were affected ($P<0.05$) by the enzyme level. A linear increase ($P<0.05$) in CP and TP contents of oat grass silage was observed with increasing enzyme level. However, a linear decrease ($P<0.05$) in NDF and ADF contents was observed with increasing enzyme level. Highest NDF and ADF contents were observed in E0, while lowest in E3 level. The DM and OM losses remained unaffected ($P>0.05$) across all enzyme levels. A linear decrease ($P<0.05$) in CP and TP losses was noticed in silage treated with increasing enzyme level. Highest NDF and ADF losses were noted in E3 which were at par with E1 and E2, while lowest in E0 which were only 37 and 36 % of highest NDF and ADF losses. Increasing enzyme level caused a linear decrease ($P<0.05$) in pH during 1st, 2nd and 3rd week of ensilation. Upon in-situ digestion kinetics it was observed that the enzyme treatment didn't affect ($P>0.05$) the extent of digestion and lag time of DM, CP, NDF and ADF and digestibility of CP, NDF and ADF and rate of DM, NDF and ADF digestion. In experiment-II, WS was treated with 4% urea and 6% molasses and was ensiled with 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme /Kg of dry matter (DM). Wheat straw was ensiled in 36 laboratory silos under Completely Randomized Design for twenty one days. Application of enzymes at the time of ensilation of WS didn't affect ($P>0.05$) the DM, CP, TP, NDF and ADF contents. Overall pH of WS ensiled with varying enzyme level ranged from 8.42 to 8.47. Enzyme treatment didn't affect ($P>0.05$) the pH of the ensiled WS. Lag time, digestion rate, in-situ digestibility and extent of digestion of DM, NDF and ADF also remained unaltered ($P>0.05$) across all the treatments. Results indicate that enzyme application at the time of ensilation can reduce the nutrient losses and fiber fractions of oat grass silage, without affecting the digestibility of fiber fraction of the silage. Whereas, enzyme application has no effect on chemical composition and digestion kinetics of WS.

CHAPTER-I

Introduction

Ruminants ferment low quality feed stuff to produce quality food for human consumption and they provide livelihood to small holders. In Pakistan, ruminants are mainly raised on pastures, ranges and seasonal fodders. Most of the available feed resources are high in fiber, which is known to limit the feed intake and digestibility (Khan et al., 2006). Energy and protein are the two major feed components but, at present ruminants are getting only 62 and 74% of their crude protein (CP) and energy requirements (Sarwar et al., 2002). Furthermore, the arable land for fodder production is constantly reducing because of human pressure for infrastructural development (Thomas and Rangnekar, 2004). Inadequate availability of quality fodder (Nisa et al., 2008) along with scarcity periods is also a major constraint in the productivity of ruminants (Sarwar et al., 2002). So, their productivity is compromised due to the deficiency of protein and energy. Fodder conservation and improvement in the nutritive value of fibrous feed stuff can bridge the gap between nutrient availability and requirements.

Ensiling the fodder during fodder availability periods can ensure the continuous supply of fodder round the year. However, fermentation losses in silage may deteriorate silage quality. During ensilation period, plant continues to respire until the anaerobic conditions are maintained. This respiration exhausts the fermentable carbohydrate contents of plant (Muck, 1988). Furthermore, intrinsic plant protease activity degrades the protein into ammonia. Protease enzyme activity can be minimized by attaining a rapid decrease in silage pH (Keady and Murphy, 1997). Lower pH also reduces the chance of mold growth and proliferation of undesirable bacterial species (Kung, 2000). Silage fermentation characteristics can be improved by the use of certain additives like fibrolytic enzymes at the time of ensilation.

Fibrolytic enzymes have been shown to improve fermentation during the ensiling of some forage crops. They efficiently hydrolyze structural carbohydrates (McHan, 1986) and thus yield more energy for lactic acid producing microbes (Stokes, 1992). Increased production of lactic acid results in more declined silage pH than that observed in untreated forage (Spoelstra et al., 1992) and thus silage stability is improved. They also have been reported to improve the aerobic stability and dry matter (DM) and fiber digestibility of Bermuda grass silage (Dean et al., 2005). Addition of fibrolytic enzymes to the silage results in degradation of cell wall components to

simpler molecules, thereby providing silage bacteria with more fermentable substrate (McDonald et al., 1991) and another advantage by degrading the cell walls of the forage, the rate and extent of digestion of silage in the rumen may be increased (Bolsen et al., 1995). Thus, the application of exogenous enzymes at the ensilation of forage may improve the quality of the resultant silage.

Because of their abundant availability, crop residues like wheat straw (WS) receive much attention as animal feed in developing countries (Khan et al., 2007). However, low digestibility, protein and high fiber contents of these residues limit their use in ruminant nutrition (Abo-Eid et al., 2007). Feeding value of these fibrous residues can be improved by employing certain physical, chemical and biological treatments and among these treatments urea treatment have been used more frequently (Sarwar et al., 2002, 2006). Ammoniation of crop residues by urea treatment has been reported to improve the digestibility, N contents and thus increase the intake of these residues by the animals (Khan et al., 2006). However, 70% of the ammonia produced from urea escapes to the environment that make this process more expensive and cause environmental pollution (Khan et al., 2007). While low pH may be helpful in improving the overall N contents of urea treated WS, as at low pH free NH3 is converted to NH+4 which is more reactive and thus bounds with fiber (Khan et al., 2006).

Enzymes have been reported to reduce the pH of grass silage (Dean et al., 2005) and maize stover silage (Sun et al., 2009). Enzymes hydrolyze the fiber into sugars that are used by silage bacteria to produce lactic acid that ultimately reduce the silage pH (McDonald et al., 1991). Organic acids have also been successfully used to reduce the pH and thus to improve the NH3 capture in urea treated WS (Sarwar et al., 2004). So the application of enzymes at the ensilation may improve the NH3 capture by lowering the pH and meanwhile reducing the fiber contents of the urea treated WS. Application of the fibrolytic enzymes at the time of ensilation of forage has been reported to reduce the fiber contents and pH of grasses (Rodrigues et al., 2001; Selmer-Olsen et al., 1993), legume (Nadeau and Buxton, 1997), whole plant (Zahiroddini et al., 2004; Adogla-Bessa et al., 1999) and maize stover silage (Sun et al., 2009). However, the research data regarding effect of enzyme application on the characteristics of urea treated WS is limited.

So, the present study was planned to examine the influence of fibrolytic enzymes on chemical composition and digestion kinetics of oat grass silage and urea-treated WS.

CHAPTER-II

Review of Literature

Fiber degrading enzyme additives for ruminants was first examined in the 1960s, as reviewed by Beauchemin and Rode (1996). Burroughs *et al.* (1960) evaluated the effect of Agrozyme® on fattening performance of cattle and reported 7% higher weight gain by animals fed diets supplemented with enzyme mixture as compared to the control. Rust *et al.* (1965) also reported a significant increase in nitrogen and energy digestibility in steers fed diets supplemented with bacterial protease as compared to the placebo. However, the effect of exogenous enzymes on the productive performance of ruminants has been actively researched during last two decades. Improvements in fermentation biotechnology have made exogenous enzymes an economical choice to improve the performance of ruminants. Now a days, the exogenous enzymes that are commercially used in animal feed industry are microbial fermentation products produced by a batch fermentation process (Cowan, 1994) and usually are of fungal (mostly Trichoderma longibrachiatum, Aspergillus niger, A. oryzae) and bacterial (mostly Bacillus spp.) origin (Pendleton, 2000). Common source organisms for enzyme production are presented in Table.2.1. Exogenous enzymes can be used as silage additive or may be fed directly to the animal.

Enzymes as Silage Additive

Several fibrolytic enzyme products evaluated as feed additives in ruminant diets were originally developed as silage additives (Feng *et al.*, 1996). Exogenous enzymes are usually used while ensiling the fodder as they act as silage preservative and improve the silage stability (Dean, 2005). Colombatto *et al.* (2003) reported that addition of fiber degrading enzymes while ensiling the forage can improve the chemical characteristics of the silage. Addition of exogenous cell wall degrading enzymes to the forage while ensiling results in reduced fiber contents of the silage (Stokes and Chen, 1994) that ultimately improve the dry matter (DM) intake (Beuvink and Spoelstra, 1994). Dean *et al.* (2008) concluded that the nutritive value and fermentation of Bermuda grass silage can be improved by the addition of fibrolytic enzymes, while ensiling the grass. He reported higher aerobic stability and lower pH of the enzyme-treated silage as

compared to the control. So the enzyme addition to the silage while ensiling the forage may minimize the nutrient losses, improve the fermentation and utilization of the silage.

Table.2.1. Common sources microbes for the production of enzymes

Enzyme	Common source organisms	Potential application
Proteases	· Aspergillus species · Bacillus species	· Potential for weakening protein
Fiber degrading enzymes (e.g., cellulase, hemicellulase, pectinase)	· Aspergillus species · Trichoderma longibrachiatum	· Improve utilization of fibrous feed stuffs
Amylases	· Aspergillus species · Bacillus species	· Improve digestion in starchy feeds

Adapted from Kung (2000)

Fermentation Losses

The objective of ensilation of fodder is to preserve nutrients efficiently; however fermentation losses in the silage leads to decreased nutritive value of the ensiled fodder. Nutritive value of the silage is dependent upon fermentation process in the silo (Charmley, 2000). Plant continues to respire after cutting until the anaerobic conditions are maintained. This respiration, on the cost of fermentable carbohydrates and oxygen produces heat and carbon dioxide (Muck, 1988) and thus decreases the fermentable carbohydrate contents in fodder. This can be avoided by maximum air elimination from the silo at the time of ensilation which helps to achieve anaerobic conditions rapidly and thus ceases the respiration. Intrinsic plant proteases are the second threat to the silage quality. Plant proteases degrade the plant protein to non-protein nitrogen contributing towards poor true protein (TP) value of silage (Kung, 2000). A rapid decrease in pH and well maintained anaerobic conditions can reduce the activity of proteases (Keady and Murphy, 1997) and chance of clostridial or mold growth. Effect of some of the silage fermentation products on silage quality and their usual amounts for different silages are presented in Table.2.2 and 2.3.

Certain additives like exogenous enzymes can affect the nutritive value of the silage by improving the fermentation. Enzymes provide the lactic acid producing bacteria with reducing sugars by hydrolyzing the plant polysaccharides which leads to the dominated growth of epiphytic bacteria and thereby increase the lactic acid production (Kung *et al.*, 2003) and reduce the silage pH. This could lead to reduced nutrient losses in the silo by inhibiting prolonged fermentation. They have also been reported to reduce the production of undesirable fermentation products like butyric acid (Adesogan *et al.*, 2004) and ammonia nitrogen (Zahiroddini *et al.*, 2004).

Dean *et al.* (2005) reported a linear decrease in DM losses in Bermuda grass silage treated with increasing levels of Biocellulase (at the rate of 7.3, 14.4 and 29 mg/kg of DM). Adogla-Bessa and Owen (1995) also reported reduced DM losses upon air exposure in wheat silage treated with Clampzyme (@ 0, 333, 667 and 1000 mL/ton of DM) as compared to the untreated silage. However, Stokes and Chen (1994) reported significantly reduced DM contents after 56 days of ensilation for the corn silage treated with enzyme mixture (Farmline International Inc., Schaumberg, IL; 264 mL/ ton of forage) as compared to the control. This loss in DM might be because of significantly reduced neutral detergent fiber (NDF) and acid

detergent fiber (ADF) contents of enzyme treated corn silage (Stokes and Chen, 1994). Xing *et al.* (2009) also reported significantly lower NDF and ADF contents for sorghum straw silage treated (@ 0.003 mg/g of fresh material) with a commercial cellulase+ hemicellulase mixture (Snow Brand Seed Ltd., Sapporo, Japan). They also reported significantly higher CP value for enzyme treated sorghum silage as compared to the control. This improved CP was might be because of reduced proteolytic activity in enzyme treated silage which reflected in significantly lower ammonia (Xing *et al.*, 2009). While, Sun *et al.* (2009) reported significantly decreasing trend in DM, CP, NDF and ADF losses for maize silage in response to the application of increasing cellulase level (0, 10 and 20 mL/ Kg), however they found that enzyme treatment significantly increase the water soluble carbohydrates (WSC) losses. Higher WSC losses was might be due to rapid growth of lactic acid producing bacteria in enzyme treated silage which lead to more consumption of WSC and resulting in rapid decline in pH which was 4.32, 4.32 and 4.15 for maize silage treated with 0, 10 and 20 mL of cellulase/ Kg, respectively in that study. This declined pH might have reduced DM, CP, NDF and ADF losses in enzyme treated silage by inhibiting prolonged fermentation (Muck, 1993).

Nutrient Composition

Enzyme addition to the forage while ensiling may affect the nutritive quality of the silage. Reduction in the fiber contents of the silage is a major effect of enzyme addition as it may increase the DM intake when fed to the animal (Stokes and Chen, 1994). Colombatto *et al.* (2004) reported significantly lower ADF contents of the enzyme-treated maize silage as compared to the control, however NDF remained unaltered. Sheperd and Kung (1996b) reported 22, 12 and 35% decrease in NDF, ADF and hemicellulose contents of maize silage treated with Cornzyme®. Similarly, Nadeau *et al.* (2000) also reported a 30% decrease in NDF contents of orchard grass silage treated with cellulase @ 10mL/Kg. While, Mandebvu *et al.* (1999) concluded that addition of fibrolytic enzymes to the Bermuda grass silage had no effect on cell wall composition and end products of silage fermentation. In contrast, Adogla-Bessaa *et al.* (1999) reported significantly decreasing cellulose, NDF and ADF contents of wheat silage treated with increasing levels (1.75, 3.5 and 15.503 L/ton of DM) of cellulase-hemicellulase mixture. Stokes and Chen (1994) also reported significantly reduced cellulose and hemicellulose after 56 days of ensilation for the corn silage treated with enzyme mixture (Farmline

International Inc., Schaumberg, IL; 264 mL/ ton of forage) as compared to the control. However, Zahiroddini *et al.* (2004) reported significantly reduced ADF but unchanged NDF contents of whole crop barley silage in response to SilagePro® (a combination of lactic acid producing bacteria and enzymes) treatment. While, Kung *et al.* (1990) reported unaltered DM, NDF and ADF contents for vetch and barley silage treated with cellulase (200 mL/25 Kg of wet forage) as compared to the untreated silage. Murray *et al.* (2007) reported unaltered NDF, ADF while reduced DM contents of lucerne silage treated with a fibrolytic enzyme mixture (applied @ 0, 2.3, 5.5 and 10.2 L/ton of DM) as compared to the untreated silage. In contrast, Rodrigues *et al.* (2001) reported significantly improved DM and CP and reduced NDF and ADF contents in rye grass silage treated with cellulase (@ 0.2 g/kg grass on as such basis) and endoxylanase (@ 0.05 g/kg grass) as compared to the untreated grass.

Reduction in fiber contents in response to enzyme treatment may be related to conversion of some fractions of fiber to the reducing sugars by the enzymes (Kung *et al.*, 2003). Nadeau *et al.* (2000) reported higher amount of total reducing sugars in orchard grass and alfalfa silage treated with cellulase (@ 10mL/Kg) as compared to the untreated silage. Stokes (1992) reported significantly increased WSC and decreased ADF contents in grass-legume forage silage treated with a commercial fibrolytic mixture (FS-01: Farmline International Ltd., Schaumberg (applied at 330 mL/ ton of forage) as compared to the untreated silage. Sheperd and Kung (1996a) observed a linear increase in glucose contents of maize silage collected at three different stages of age in response to increasing levels of Cornzyme® (0, 1, 10 and 100 times of recommended dose) application. In another study, Sheperd and Kung (1996b) also reported higher glucose at 56 and 105 days of ensilation in maize silage treated with Cornzyme® (@ 220 mL/ ton of DM) as compared to the untreated silage. Likewise, Meeske *et al.* (1999) reported higher WSC in *Digitaria eriantha* silage treated with enzyme and bacterial inoculant. However, Kung *et al.* (1990) observed unaltered WSC in vetch and barley silage treated with cellulase (200 mL/25 Kg of wet forage) as compared to the untreated silage. Colombatto *et al.* (2004) reported significantly reduced WSC in maize silage treated with enzyme as compared to the untreated silage. However, Selmer-Olsen (1994) concluded that cellulase+ hemicellulase treatment increases the total WSC production in the silage of herbage from mixed pastures of timothy, meadow fescue and red clover. The variable effect of enzyme addition on silage composition

may be due to crop-specific nature of inoculants and enzymes (Nadeau and Buxton, 1997) and forage stage of maturity (Van Vuuren *et al.*, 1989).

Table.2.2. Common end products of silage fermentation

Items	Effect(s)	Actions(s)
pH	+	Low pH inhibits bacterial activity
Lactic acid	+	Inhibits bacterial activity by lowering pH
Acetic acid	-	Associated with undesirable fermentations
	+	Inhibits yeasts responsible for aerobic spoilage
Butyric	-	Associated with protein degradation, toxin formation, and large losses of DM and energy
Ethanol	-	Indicator of undesirable yeast fermentation and high dry matter losses
Ammonia	-	High levels indicate excessive protein breakdown
Acid detergent insoluble nitrogen	-	High levels indicate heat-damaged protein and low energy contents

Adapted from Kung (2000)

Table.2.3. Amounts of common fermentation end products in various silages

Items	Alfalfa Silage, 30 - 35% DM	Alfalfa Silage, 45 - 55% DM	Grass Silage, 25 - 35% DM	Corn Silage, 35 - 40% DM	High Moisture Corn, 70 - 73% DM
pH	4.3 - 4.5	4.7 - 5.0	4.3 - 4.7	3.7 - 4.2	4.0 - 4.5
Lactic acid, %	7 - 8	2 – 4	6 – 10	4 - 7	0.5 - 2.0
Acetic acid, %	2 - 3	0.5 - 2.0	1 – 3	1 - 3	< 0.5
Propionic acid, %	< 0.5	< 0.1	< 0.1	< 0.1	< 0.1
Butyric acid, %	< 0.5	0	<0.5	0	0
Ethanol, %	0.5 - 1.0	0.5	0.5 - 1.0	1 - 3	0.2 - 2.0
Ammonia-N, % of crude protein	10 – 15	< 12	8 – 12	5 – 7	< 10

Adapted form Kung (2000)

Table.2.4. Change in fiber contents of forages ensiled with enzymes

Source	Change in fiber concentration (g/ Kg dry matter)	
	Neutral detergent fiber	**Acid detergent fiber**
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.* (2001)	-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 Adesogan (2005)

Silage pH

Rapid decline in silage pH can prevent the plant protein from degradation and mold and undesirable microbial species growth (Kung, 2000). It is well documented that addition of enzymes to the silage decreases the silage pH. Adogla-Bessa *et al.* (1999) treated whole wheat crop silage with cellulase-hemicellulase mixture while ensiling and found reduced pH of the silage as compared to the untreated silage. Likewise, Colombatto *et al.* (2004) reported a reduction in pH of corn silage in response to enzyme addition. This reduced silage pH conserves the water soluble carbohydrates and prevents the deamination by inhibiting prolonged fermentation (Muck, 1993). Dean *et al.* (2005) reported a linear decrease in pH of Bermuda grass silage treated with increasing levels (0.65, 1.3, and 2.6 g/kg of DM) of Promote NET (Pr; Cargill Corp., St. Louis, MO). Reduction in pH in response to enzyme treatment may be due to availability of fermentable carbohydrates and increased growth of epiphytic bacteria (Kung *et al.*, 2003), this release of fermentable sugars increase the rate and extent of lactic acid production in the silage (Kozelov *et al.*, 2008). Higher lactic acid production in enzyme treated silage results in declined pH (Spoelstra *et al.*, 1992) that improves the silage stability. However, Zahiroddini *et al.* (2004) reported unchanged pH and lactic acid contents for whole crop barley silage treated with SilagePro® (a combination of lactic acid producing bacteria and enzymes) as compared to the untreated silage. While, Stokes (1992) observed higher lactic acid production in grass-legume forage silage treated with a commercial fibrolytic mixture (FS-01: Farmline International Ltd., Schaumberg (applied at 330 mL/ ton of forage) as compared to the untreated silage. Whereas, Chen *et al.* (1994) reported unaltered lactic acid and pH for corn silage treated with Alfazyme (applied @ 220 mL/ton: Farmline International Inc., Schaumburg) as compared to the untreated silage. For maize silage treated with maize-all® (@ 10g/ ton), Donmez *et al.* (2003) reported significantly increased lactic acid production as compared to the untreated or silage treated with formic acid (0.05%), however, pH remained unaltered across all treatments.

Sheperd and Kung (1996a) observed a quadratic trend in pH reduction of maize silage collected at three different stages of age in response to increasing levels of Cornzyme® (0, 1, 10 and 100 times of recommended dose) application. Stokes (1992) also reported significantly decreased pH of grass-legume forage silage treated with a commercial fibrolytic mixture (FS-01: Farmline International Ltd., Schaumberg (applied at 330 mL/ ton of forage) as compared to the

untreated silage, but the lactic acid production remained unaffected. Stokes and Chen (1994) also reported unchanged lactic acid contents after 56 days of ensilation of the corn silage treated with enzyme mixture (Farmline International Inc., Schaumberg, IL; 264 mL/ ton of forage) as compared to the control. Whereas, Sun *et al.* (2009) observed decreasing trend in pH with increasing lactic acid contents for maize silage in response to the application of increasing cellulase level (0, 10 and 20 mL/ Kg). Nadeau *et al.* (2000) reported significantly higher lactic acid and total acid contents and lower pH in orchard grass and alfalfa silage treated with cellulase (@ 10mL/Kg) as compared to the untreated silage. Similarly, Meeske *et al.* (1999) also reported higher lactic acid while lower pH in *Digitaria eriantha* silage treated with enzyme and bacterial inoculant. In contrast, Sheperd and Kung (1996b) observed unchanged pH at 56, 105 and 196 days of ensilation in maize silage treated with Cornzyme® (@ 220 mL/ ton of DM) as compared to the untreated silage. Kung *et al.* (1990) also reported unaltered lactic acid and pH for vetch and barley silage treated with cellulase (200 mL/25 Kg of wet forage) as compared to the untreated silage. Similarly, Murray *et al.* (2007) reported unaltered lactic acid and pH of lucerne silage treated with a fibrolytic enzyme mixture (applied @ 0, 2.3, 5.5 and 10.2 L/ton of DM) as compared to the untreated silage. However, Rodrigues *et al.* (2001) reported significantly reduced pH and increased lactic acid production in rye grass silage treated with cellulase (@ 0.2 g/kg grass on as such basis) and endoxylanase (@ 0.05 g/kg grass) as compared to the untreated grass.

Variability in lactic acid production and pH in response to enzyme treatment may be related to the forage type and maturity. By conducting seven experiments with silage of herbage from mixed pastures of timothy, meadow fescue and red clover treated with cellulase+ hemicellulase or bacterial inoculant, Selmer-Olsen (1994) concluded that enzyme treatment has more extensive potential for decreasing pH and increasing lactic acid contents of herbage with less fermentable carbohydrates than those with more sugar contents. Adogla-Bessa and Owen (1995) reported significantly deceased pH and higher lactic acid production for wheat silage treated with Clampzyme (@ 0, 333, 667 and 1000 mL/ton of DM) as compared to the untreated silage and found that the enzyme treatment has significant interaction with growth stage of the forage. However, sometimes pH doesn't seem to be influenced by lactic acid contents. Donmez *et al.* (2003) used molasses (5%), formic acid (0.05%), maize-all® (@ 10g/ ton) or no additive to

ensile corn silage. They found highest lactic acid contents ($P<0.05$) in molasses treated silage (4.39%) followed by enzyme treated (3.60%), formic acid (2.60%) and untreated (1.73%) treated silage, but the results for pH were statistically non-significant for all treatments.

Table.2.5. Effect of exogenous enzyme on silage pH

Source	Forage type	pH		Lactic Acid (% Higher than control)
		Control	Enzyme-treated	
Kung et al. (1990)	Barley	4.4	4.3	13
Adogla-Bessa et al. (1999)	Wheat	4.9	4.4	40
Nadeau et al. (2000)	Orchard grass and Alfalfa	4.5	4	45
Zahiroddini et al. (2004)	Barley	3.9	3.7	10

Silage Ammonia Nitrogen

Ammonia nitrogen concentration is the measure of protein degradation in the silage. Higher proteolysis results in more conversion of protein to ammonia which ultimately causes

poor silage intake by the animal (Charmley, 2001). Dean *et al.* (2005) reported a linear decrease in ammonia N in Bermuda grass silage treated with increasing levels of Biocellulase A-20 (at the rate of 7.3, 14.4 and 29 mg/kg of DM). However, Nadeau *et al.* (2000) reported higher ammonia production in orchard grass and alfalfa silage in response to the cellulase treatment during ensiling. While, Meeske *et al.* (1999) reported lower ammonia N in *Digitaria eriantha* silage treated with enzyme and bacterial inoculant. Sheperd and Kung (1996b) also reported reduced ammonia after 56 days of ensilation in maize silage treated with Cornzyme® (@ 220 mL/ ton of DM) as compared to the untreated silage. Zahiroddini *et al.* (2004) reported significantly reduced ammonia N for whole crop barley silage in response to SilagePro® (a combination of lactic acid producing bacteria and enzymes) treatment. However, Stokes (1992) reported unchanged ammonia N in grass-legume forage silage treated with a commercial fibrolytic mixture (FS-01: Farmline International Ltd., Schaumberg (applied at 330 mL/ ton of forage) as compared to the untreated silage. Kung *et al.* (1990) also reported unaltered ammonia N for vetch and barley silage treated with cellulase (200 mL/25 Kg of wet forage) as compared to the untreated silage. Likewise, Murray *et al.* (2007) reported unaltered ammonia N for lucerne silage treated with a fibrolytic enzyme mixture (applied @ 0, 2.3, 5.5 and 10.2 L/ton of DM) as compared to the untreated silage.

Chen *et al.* (1994) reported significantly decreased ammonia in corn silage treated with Alfazyme (applied @ 220 mL/ton: Farmline International Inc., Schaumburg) as compared to the untreated silage. However, Rodrigues *et al.* (2001) reported lower ammonia nitrogen for untreated grass silage as compared to the grass treated with cellulase (@ 0.2 g/kg grass on as such basis) and endoxylanase (@ 0.05 g/kg grass) as compared to the untreated grass. Selmer-Olsten (1994) conducted seven experiments on silage of herbage from mixed pastures of timothy, meadow fescue and red clover treated with cellulase+ hemicellulase or bacterial inoculant and concluded that enzyme treatment results in reduced ammonia N of the silage. Van Vuuren *et al.* (1989) also reported significantly reduced ammonia N concentration for silage of 17% *Trifolium repens* and 83% *Lolium perenne* herbage in response to commercial mixture of fibrolytic enzyme treatment. Selmer-Olsen *et al.* (1993) treated ryegrass with cell wall degrading enzymes (@ 0, 0·125, 0·250, 0·500 and 0·750 mL/Kg) and reported significantly reduced silage ammonia N for enzyme treated silage as compared to the untreated ryegrass. Whereas, Meeske *et*

al., (2002) reported similar ammonia N for round bale oat silage treated with or without Sill-All (Alltech®) together with amylase, cellulase and hemicellulase (@ 10g/ton of fresh material).

Variable responses of silage ammonia N towards enzyme treatment can be related to the differences in lactic acid production and pH, as ammonia production is dependent on the pH of the silage (Muck, 1993). Schmidt *et al.* (2001) observed higher ammonia N in SSF® (fibrolytic crude enzyme mixture by Alltech®) treated alfalfa silage when pH was lower. They further stated that higher ammonia N was due to lower lactic acid production that was unable to influence pH. However, Koc *et al.* (2008) reported significantly reduced pH while unaltered ammonia N for maize silage treated with amylase (Maize-All, Alltech®) and bacterial inoculant (@ 0, 0.15, 0.30, 0.60 g /Kg DM). This may be attributed to higher WSC contents of maize, as enzymes are more effective to improve the silage characteristics of forages with less WSC as compared to the forages with higher fermentable carbohydrates (Selmer-Olsen, 1994). Thus silage can be preserved well in context of protein contents by the application of enzymes at the time of ensilation.

In-vitro Gas Production

In-vitro gas production (GP) production is a measure of extent and rate of fermentation of the silage. Van Der Meer *et al.* (1988) concluded that the GP technique developed by Menke *et al.* (1979) was capable to detect the changes in the silage when treated with fibrolytic enzymes. Eun *et al.* (2006) also reported that in-vitro GP and DM degradation were helpful in determining the changes in the substrate availability due to the addition of enzymes. Colombatto *et al.* (2004) reported higher GP rate in maize silage in response to exogenous enzyme addition in early fermentation stage. Similarly, Kozelov *et al.* (2008) reported higher GP (61.7 versus 51.3 mL/ 100 mg of silage) for alfalfa silage ensiled with cellulase (@ 5Kg/ ton) as compared to the untreated silage. For maize silage ensiled with increasing cellulase levels (0, 10 and 20 mL/ Kg), Sun *et al.* (2009) observed increasing trend in accumulative GP. Wang *et al.* (2002) treated whole crop barley and corn silage with xylanase and β-glucanase (10 mL solution of water and enzyme (obtained by dissolving 150g of enzyme powder in 1L of water)/ Kg of DM) and reported significantly higher 2, 12, 24 and 48 h GP for enzyme treated corn and barley silage as compared to the untreated silage.

Lv *et al.* (2005) reported higher GP for rice straw ensiled with wheat bran and Strawzyme (fibrolytic enzyme mixture; 1300g/ton of DM) as compared to the rice straw ensiled with wheat bran alone. Tang *et al.* (2008) reported a linear increase in accumulative GP for maize stover, maize stover silage, WS and rice straw treated with increasing levels (0, 2.5, 5 and 7.5 g/Kg) of a commercial enzyme (Yingheng Biotech Ltd, Guangdong Province, China) having cellulase and xylanase activity. They observed quadratic responses of lag time towards increasing level of enzyme for all forages except for the rice straw for which linear trend was observed. However, Wang *et al.* (2004) reported that addition of fibrolytic enzyme to WS resulted in higher GP up to 8h of fermentation. Jalilvand *et al.* (2008) also reported higher GP in enzyme treated WS at 6 and 12h of fermentation.

This indicates that addition of exogenous enzymes to silage improves the fermentation efficiency in initial stages of GP which might be attributed to decreased lag time and provision of soluble carbohydrates resulting in rapid growth of the microbes (Forsberg *et al.*, 2000). Beuvink and Spoelstra (1994) reported lower initial lag phase for the grass ensiled with cellulase as compared to the control. However, they reported unaltered accumulative GP but rapidly degradation fraction was significantly higher for the grass silage treated with enzyme. Higher rates of initial GP may be related higher rapidly degradable fiber fraction. This phenomenon may contribute towards nutrient synchronization at ruminal level by providing carbon skeleton for the ammonia fixation more rapidly. Thus it can be concluded that inclusion of enzymes in silage enhances the initial rate of fermentation by increasing the rapidly fermentable fraction.

Digestion Kinetics

Reports regarding the effect of enzyme application prior to ensiling on the digestion kinetics of the silage are inconsistent. Enzymes may not alter the digestion rates and lag time but proportion of slowly or rapidly degradable fractions may be affected. Zahiroddini *et al.* (2004) reported unaltered DM and NDF lag time and digestion rate for whole crop barley silage in response to SilagePro® (a combination of lactic acid producing bacteria and enzymes) treatment,

however, soluble NDF fraction was significantly higher (81 versus 4 g/Kg of NDF) for enzyme treated silage as compared to control. Chen *et al.* (1994) reported unaltered DM and NDF digestion rates for corn and hay crop silage treated with Alfazyme (applied @ 220 mL/ton: Farmline International Inc., Schaumburg) as compared to the untreated silage, however slowly degradable fractions of DM and NDF were significantly higher (60.72 versus 55.42 and 93.55 83.73 %, respectively) in untreated hay crop silage. Sheperd *et al.* (1995) reported higher initial hour (up to 48th hour) *in-vitro* NDF degradability for alfalfa silage ensiled with or without the application of Alfazyme^{TM} (@ 3L/ ton of material), however after 36-48 hours degradability was higher for untreated silage.

Mandebvu *et al.* (1999) unaltered lag time and digestion rate for DM and ADF and concluded that addition of fibrolytic enzymes to the Bermuda grass silage had no effect on digestion kinetics. Zhu *et al.* (2011) ensiled Rhodes grass and Guinea grass with or without the addition of cell wall degrading enzymes (@ 50mg/ Kg of fresh material). They reported unaltered rate of degradation, extent of digestion and lag time for both silages treated with or without enzymes. However, rapidly degradable DM fraction was significantly higher for silages ensiled with enzyme as compared to the control. Weinberg *et al.* (1993) also reported unaltered ruminal DM digestion for wheat and rye grass ensiled with or without the application of cellulase or lactic acid producing bacteria. Zhu *et al.* (1999) also reported similar *in-situ* digestion rate and lag time for Italian rye grass and Lucerne ensiled with or without the application (@ 50mg/ Kg) of fibrolytic enzymes. However, rate and extent of NDF digestion was significantly lower for the silage treated with enzyme. Jaakola *et al.* (1991) reported significantly higher NDF and ADF ruminal retention time in dairy cattle for timothy grass ensiled with fibrolytic enzymes as compared to the control.

Nutrient Digestibility

Addition of enzyme to the silage while ensiling can improve the digestibility of the resultant silage. Colombatto *et al.* (2004) reported significantly improved *in-vitro* organic matter (OM) digestibility of maize silage treated with enzyme as compared to the untreated silage. While, Mandebvu *et al.* (1999) observed that enzyme addition to silage has no effect on digestibility of silage. Likewise, Nadeau *et al.* (2000) reported unaltered *in-vitro* DM and NDF

digestibility of enzyme-treated alfalfa silage. Kozelov *et al.* (2008) also reported unaltered *in-vitro* DM digestibility for alfalfa silage ensiled with or without 5 Kg/ ton of cellulase. While, Sheperd and Kung (1996b) observed decreased NDF digestibility of maize silage treated with Cornzyme®. In contrast, Meeske *et al.* (1999) reported significantly higher OM *in-vitro* digestibility (57.4 versus 54.6%) for *D. eriantha* silage ensiled with or without 10^6 colony forming units (CFU) of lactic acid producing bacteria with fibrolytic enzyme mixture. Xing *et al.* (2009) also reported significantly higher DM and NDF *in-vitro* digestibility (54.3 versus 52.2 % and 45.8 versus 43.25 %, respectively) for sorghum straw silage ensiled (@ 0.003 mg/g of fresh material) with or without commercial cellulase+ hemicellulase mixture (Snow Brand Seed Ltd., Sapporo, Japan). While, Kung *et al.* (1990) reported similar *in-vitro* NDF digestibility for vetch and barley silage treated with cellulase (200 mL/25 Kg of wet forage) as compared to the untreated silage. Sheperd *et al.* (1995) also reported similar *in-vitro* NDF digestibility for alfalfa silage ensiled with or without the application of Alfazyme^{TM} (@ 3L/ ton of material).

Mandebvu *et al.* (1999) found that enzyme+ microbial application at the time of ensilation has no affect on *in-vitro* and *in-situ* DM and NDF of Bermuda grass silage. Adogla-Bessa and Owen (1995) also reported unaltered *in-vitro* OM digestibility for wheat silage treated with increasing levels of Clampzyme (@ 333, 667 and 1000 mL/ton of DM) as compared to the untreated silage. . Sheperd and Kung (1996b) reported similar OM, CP, NDF and ADF digestibility in growing lambs fed diets containing maize silage ensiled with or without Cornzyme®. However, Patterson *et al.* (1997) reported significantly higher DM, OM, and energy digestibility in sheep fed grass silage ensiled with enzyme preparation (SIL-ALL, Alltech UK; @ 3 L/ton) as compared to the control. Jaakola *et al.* (1991) also reported significantly higher cellulose digestibility by dairy cattle for timothy grass ensiled with fibrolytic enzymes as compared to the formic acid treated silage.

Decreased or unaltered digestibility in response to enzyme addition might be related to less digestible cell wall remaining in the silage after hydrolysis of fiber by enzyme during ensiling (Nadeau *et al.*, 1996). Tang *et al.* (2008) reported a linear increase in *in-vitro* OM and DM digestibility of rice straw and WS when treated with increasing levels (0, 2.5, 5 and 7.5 g/Kg DM) of concentrated enzyme mixture (Yingheng Biotech Ltd, Guangdong Province, China) having cellulase and xylanase activity. However, Jacobs *et al.* (1991) reported unaltered nutrient

digestibility by sheep fed perennial ryegrass ensiled at different levels of DM with or without formic acids and enzymes.

Animal Responses

Dry Matter Intake

Fermentation characteristics of silage directly influence the silage intake. Higher ammonia and butyric acid production in silo may result in reduced palatability of silage ultimately reducing the silage intake by the animal (Cushnahan *et al.* 1995). Studies have shown that ensilation of forage with enzyme results in lower production of butyric acid (Adesogan *et al.*, 2004; Adogla-Bessa *et al.*, 1999) and ammonia nitrogen (Dean *et al.*, 2005; Zahiroddini *et al.*, 2004). On the other hand, researchers have also observed that enzyme treatment results in lower silage pH (Sun *et al.*, 2009; Kozelov *et al.*, 2008) which may lead to lower DM intake by reducing ruminal pH and depression in cellulolytic activity (Carmley, 2001). However, Rooke (1995) reported that no relationship exists between ruminal and silage pH in sheep fed grass silage treated with varying acid concentration. Despite of these theories, reports regarding effect of ensiling forage with enzyme on intake are in-consistent. Meeske *et al.* (1999) reported significantly higher DM intake (1848 versus 1540 g/day) by mature Marino rams fed *D. eriantha* silage ensiled with or without 10^6 colony forming units (CFU) of lactic acid producing bacteria with fibrolytic enzyme mixture. Patterson *et al.* (1997) also reported higher DM intake by dairy cattle fed grass silage ensiled with enzyme preparation (SIL-ALL, Alltech UK; @ 3 L/ton) as compared to the control. However, Jacobs *et al.* (1991) reported similar voluntary intake by sheep fed perennial ryegrass ensiled with or without enzyme preparation. Unaltered intake was might be attributed to similar nutrient digestibility for the enzyme treated or untreated silage (Jacobs *et al.*, 1991). However, Stokes (1992) reported higher DM intake by Holstein cows fed mixed grass-legume (50:50) forage ensiled with a fibrolytic enzyme preparation (FS-01: Farmline International Ltd., Schaumberg; applied at 330 mL/ ton of forage) as compared to those fed untreated silage. Higher intake might be related to more reduction of NDF contents in silo for enzyme treated silage. Thus enzyme application at the time of ensilation can result in better DM intake of the forage by reducing ammonia and butyric acid production and reducing the fiber contents of the silage.

Production Performance

Silage quality can affect the animal productive performance. Enzymes have been reported the nutritive value of the silages by reducing nutrient losses (Sun *et al.*, 2009; Xing *et al.*, 2009). This could lead to better animal performance of the animals fed silages ensiled with enzymes. Meeske *et al.* (2002) reported higher milk production by the Jersey cows fed big round bail oat silage ensiled with an enzyme product having cellulase, hemicellulase and amylase activity as compared to the animals receiving untreated silage. They also reported significantly decreased milk urea nitrogen and unaltered milk fat in response to enzyme treated silage. Reduction in milk urea nitrogen might be related to efficient utilization of dietary protein by cows receiving enzyme treated silage. However, Sheperd and Kung (1996) reported unaltered milk production for cows fed diets containing corn silage ensiled with or without the application of Cornzyme®. Patterson *et al.* (1997) also reported similar milk production for dairy cattle fed grass silage ensiled with or without enzyme preparation (SIL-ALL, Alltech UK; @ 3 L/ton). However, Stokes (1992) reported higher milk production and milk fat for Holstein cows fed mixed grass-legume (50:50) forage ensiled with a fibrolytic enzyme preparation (FS-01: Farmline International Ltd., Schaumberg; applied at 330 mL/ ton of forage) as compared to those fed untreated silage. Higher milk fat might be related to increased fiber digestibility in response to enzyme application. In conclusion, ensilation of forage with enzymes may result in better nutritive value of the silage and thus may improve the performance of animals, however sometimes the effect is lacking which might be related to less digestible fiber fraction remaining in enzyme treated silages.

Direct-fed Enzymes and Animal Responses

Ruminants have got an exceptional quality of converting low quality fibrous feed stuff into supreme quality products. This is because of microbial fermentation in the rumen. However, only 10-35% of the total energy intake is captured as net energy because 20-70% of the cellulose may not be digested in the digestive tract of the animal (Varga and Kolver, 1997). Significant improvements in forage digestibility have been achieved through different strategies. Despite of these improvements the forage digestibility continues to limit the DM intake and thus resulting in extensive nutrient excretion by the livestock (Beauchemin *et al.*, 2003). Addition of exogenous

enzymes to the diet can potentially improve the cell wall digestion and efficiency of feed utilization in ruminants.

Dry Matter Intake

Direct-fed exogenous enzymes have been shown to increase the DM intake. Feng *et al.* (1996) concluded that addition of fibrolytic enzymes to grass hay has a potential to enhance intake. He reported significantly higher DM intake for grass treated with lower enzyme level prior to feeding. Higher DM intake might be attributed to more palatability due to pre-ingestive sugar release by the enzyme (Adesogan, 2005). However, Reddish and Kung (2007) added an enzyme mixture with cellulase and xylanase activity (Alltech Inc., Nicholasville, KY) to the diet of lactating cows @ 10g/cow/d and observed no effect on DM intake. While, likewise, Meeske *et al.* (1999) also reported significantly higher DM intake in rams fed *Digitaria eriantha* silage treated with enzyme containing lactic acid bacterial inoculant. Improved DM intake in response to enzyme-treated feed might be due increased hydrolytic capacity of the rumen which indirectly reduces gut fill and thus enhancing feed intake (Adesogan, 2005).

Digestibility

Enzymes addition to forage diets can improve digestibility of the feed (Beauchemin *et al.*, 1995). Feng *et al.* (1996) reported higher in-vivo DM and NDF digestibility for grasses treated with enzyme as compared to the control. Likewise, Meeske *et al.* (1999) also reported significantly higher DM digestibility in rams fed *Digitaria eriantha* silage treated with enzyme containing lactic acid bacterial inoculant. Likewise, Iwaasa *et al.* (1997) applied an enzyme product (Xylanase B, Biovance Technologies Inc., Omaha, NE) to 95% barley based finishing cattle diet and observed 5% higher DM digestibility higher than control (Iwaasa *et al.*, 1997). Krause *et al.* (1998) also applied similar enzyme product (Xylanase B, Biovance Technologies Inc., Omaha, NE) to the high concentrate diet and reported 28% increase in ADF digestibility. Higher digestibility might be due to synergistic effect of enzyme on ruminal microflora. Nsereko *et al.* (2002) concluded that treating the diet of cow with an enzyme from *T. longibrachiatum* increase the no of ruminal bacteria that utilize hemicellulose or secondary products of cellulose digestion. Giraldo *et al.* (2007) also observed that treating a high forage substrate with a cellulase produced by *T. longibrachiatum* increased the no of cellulolytic bacteria in Rusitec fermenters.

Furthermore, exogenous enzymes release sugars from the fiber in the rumen that help the microbes to get attached to their substrate through chemotaxis (Newbold, 1997). However, Reddish and Kung (2007) reported no effect of enzyme mixture on in-vitro digestion of TMR even when added in high doses and also observed unaltered nutrient digestion in lambs fed diets treated with enzyme mixture. Whereas, Colombatto *et al.* (2007) treated alfalfa stem with six levels (0, 0.51, 1.02, 2.55, 5.1, and 25.5 µl/g of alfalfa stem) of a commercial enzyme product (Liquicell 2500, Specialty Enzyme and Biochemicals, Fresno, CA, USA) and reported a linear increase in the in-vitro OM digestibility with increasing enzyme levels.

The varying effect of enzyme addition on digestibility might be due to differences in forage type or enzyme product. Jalilvand *et al.* (2008) concluded that responses to the levels of enzyme addition differ with the forage type and the activity of enzyme. Wallace *et al.* (2001) used six enzyme products to observe the relationship between enzyme activities and in vitro gas production using grass and corn silage and reported a significant positive correlation between cellulase activity and gas production from grass silage. Colombatto *et al.* (2002) applied 23 commercial enzyme products (assayed for 39°C and pH 6.0) to evaluate the effect of enzyme activity and the in vitro degradation of feeds. The enzyme products were. He observed that the five and nine of the 23 products significantly improved the 18-h degradation of alfalfa hay and corn silage, respectively. He further stated that the relationship between xylanase activity and feed digestion was significant and was positive with alfalfa hay while negative with corn silage. Thus exogenous enzyme addition to the diet of the ruminants can improve digestibility by improving the ruminal fermentation and this improvement may reflect in more ingestive activity by the animal but the effect may be variable depending upon the level and activity of enzyme and forage type.

Table.2.6. Effect of direct-fed enzymes on intake and digestibility

Items	Bowman *et al.* (2002)		Hristov *et al.* (1998)		Beauchemin *et al.* (2000)		Hussain 2009	
	C	E	C	E	C	E	C	E
Dry matter intake (Kg/ day)	23.6	23.7	9.04	9.11	20.46[b]	22[a]	NA	NA
Nutrient digestibility, %								
Dry matter	65.1[b]	72.6[a]	80.2	81.4	64.7[b]	67.3[a]	64.3[b]	68.8[a]
Crude protein	NA	NA	75	77.2	NA	NA	NA	NA
Neutral detergent fiber	44.3[b]	55.6[a]	70	68.3	43.1[b]	44.2[a]	57.6[b]	62.9[a]
Acid detergent fiber	43.6[b]	55.6[a]	NA	NA	37.6	42.5	NA	NA

[a, b] Whiten a study means in the same row sharing different superscripts differ ($P < 0.05$).

C= Control. E= Enzyme-treated. NA= Not available.

Milk Production

Use of exogenous feed enzymes is more pronounced in dairy cattle. Beauchemin *et al.* (2003) reviewed the effect of exogenous feed enzymes on DM intake and milk yield and reported that across 20 studies and 41 treatments the average increase in DMI was 1.0 ± 1.3 kg/d and the average increase in milk yield was 1.1 ± 1.5 kg/d (3.4% ± 4.7). Lewis *et al.* (1999) applied a cellulase/xylanase mixture (Finn Feeds Int.; supplying 1 mL/kg of total mixed ration on DM basis) to the forage and reported 16% more milk production by cows in early lactation as compared to the control. Rode *et al.* (1999) treated the concentrate portion of the diet with an enzyme product (Promote, Biovance Technologies Inc., Omaha, NE; supplying 1.3 g/kg of TMR on a DM basis) and reported a 3.6 kg/d increase in milk production for cows in early lactation. Likewise, Yang *et al.* (2000) added an enzyme mixture (Biovance Technol, Omaha, NE) to the concentrate portion of the diet of cows in early lactation and observed 5.9% more milk yield, however no effect was observed when similar enzyme was added to TMR. While, Reddish and Kung (2007) added an enzyme mixture with cellulase and xylanase activity (Alltech Inc., Nicholasville, KY) to the diet of lactating cows @ 10g/cow/d and observed no effect on DM intake, milk production and composition. Whereas, Titi (2003) and Ahn *et al.* (2003) reported improvements in milk production by the addition of similar enzyme product in the lactating cows. The inconsistent effects of enzyme addition on milk production might be due to differences in levels of enzyme used and physiological state of the animal. Kung *et al.* (2000) used increasing levels of an enzyme product (Finn Feeds Int. @ 0, 1 and 2.5mL/Kg of TMR) and observed higher milk production in cows fed diets containing lower level of enzyme as compared to the control and the cows supplemented with higher level of enzyme. He further stated that a non-linear response to enzyme addition exists in dairy cattle and it is possible to over supplement. Production responses to enzymes addition in the diet are expected highest in the situations in which cell wall digestion is compromised and when the first limiting nutrient is energy (Beauchemin *et al.,* 2003). In early lactation, when the fiber digestibility is compromised due to high concentrate diets fibrolytic enzymes can improve the digestibility and thus milk production (Rode *et al.,* 1999). So addition of the exogenous enzyme to the diets of animal may bridge the gap between actual and potential production of the lactating animal.

Growth Performance

Direct fed exogenous enzymes may improve the growth performance of ruminants by improving the efficiency feed utilization, but the results are inconsistent. Iwaasa *et al.* (1997) applied an enzyme product (Xylanase B, Biovance Technologies Inc., Omaha, NE) to a 95% barley based finishing cattle diet and observed improved efficiency of feed utilization by 12% which was due to better digestibility that was 5% higher than control. Zahiroddini *et al.* (2004) observed 4.8% higher average daily gain (ADG) in steers fed enzyme-treated whole crop barley silage. McAllister *et al.* (1999) reported 10% increase in ADG in response to the addition of an enzyme product (Finnfeeds Int. Ltd.,Marlborough, U.K.) @ 3.5 L/t of DM to both the forage (ryegrass silage; 30% of the diet) and grain (barley, 70% of the diet) portions of the diet. However, ZoBell *et al.* (2000) reported no effect on weight gain when the same enzyme product (Finnfeeds Int. Ltd., Marlborough, U.K.) was added to a high-grain barley-based feedlot finishing diet containing 17% forage (DM basis). While, Zinn and Salinas (1999) reported 6% higher ADG in steers fed diets treated with fibrolytic enzymes. Addition of enzymes improves the NDF digestibility and thereby enhances the DM intake and growth performance (Zinn and Salinas, 1999).

The variability in growth performance in response to direct fed enzymes might be related to differences in enzyme levels in the diets. Beauchemin *et al.* (1995) observed increased average daily gain (24-30%) and improved digestibility in beef cattle fed alfalfa hay with lower levels of enzyme (0.25 to 1mL/Kg of DM) and reported that higher level of enzyme (2 and 4mL/Kg of DM) were not that effective.

Method and Level of Enzyme Application

Certain factors like level of enzyme used, method adopted for enzyme application and physiological stage of the animal can determine the effect of enzyme application on the animal performance. Excessive or insufficient supplementation of exogenous enzymes may result in unaltered responses. Nsereko *et al.* (2002) noticed a quadratic trend in total ruminal bacterial counts in response to increasing level of an enzyme from *Trichoderma longibrachiatum* applied to the dairy cattle ration. However, Jalilvand *et al.* (2008) concluded that responses towards enzyme addition level are dependent upon forage type.

Method through which the enzyme is applied to the animal diet is an important factor affecting exogenous enzyme action. Applying fibrolytic exogenous enzymes in a liquid form onto feeds prior to consumption can have a positive effect on animal performance (Kung *et al.*, 2000; Yang *et al.*, 2000). Contrarily, infusion of enzymes directly into the rumen has not been reported be effective (McAllister *et al.*, 1999; Sutton *et al.*, 2001). This indicates that, enzymes may have a pre-ingestive attack on plant fiber contents and/or their binding to the feed may enable them to resist proteolysis in rumen. Lewis *et al.* (1996) reported increased NDF digestibility in response to an enzyme solution which was applied to hay prior to feeding as compared enzyme applied immediately before feeding. Similar findings were reported by Colombatto, (2000) in an *in-vitro* study.

Characteristics of feed may also determine the effect of enzyme on feed utilization. Effect of exogenous enzymes may likely to be more when they are applied to moist feeds as compared to feeds having lower moisture. This is may be due to requirement of water for the hydrolysis of complex polymers. However, some enzymes have greater effect when they are applied to dry forages in a liquid form as compared to the wet forages. Feng *et al.* (1996) treated fresh and wilted grass with an enzyme solution and observed no effect; however, in response to application of enzyme solution to dried grass, they found higher DM and fiber digestibility. Likewise, Yang *et al.* (2000) observed higher milk production and nutrient digestibility in response to enzyme application to concentrate portion of diet as compared to their direct application to total mixed ration. However, Phipps *et al.* (2000) observed no difference among addition of an enzyme to concentrate portion or total mixed ration, but results were also similar for the untreated diet. Yang *et al.* (1999) reported that applying enzyme to dried forage or to both dried forage and concentrate yields similar results. However, other researchers reported that addition of enzyme to the concentrate portion of the diet is more effective (Rode *et al.*, 1999; Yang *et al.*, 2000).

Table.2.7 Effect of direct-fed enzymes on performance of beef cattle

Items	Control	Enzyme level		Reponses
		1×	2×	
Beauchemin *et al.* (1997)				
No. of animals	10	9	—	—
Dry matter intake, Kg/d	9.99	9.53	—	−5%
Average daily gain, Kg/d	1.43	1.52	—	+6%
Feed: Gain	7.11[e]	6.33[d]	—	−11%
Iwaasa *et al.* (1997)				
No. of animals	10	10	10	—
Dry matter intake, Kg/d	10.6	9.8	9.8	−8%
Average daily gain, Kg/d	2.0	2.1	2.2	+1%
Feed: Gain	5.2[g]	4.9[g]	4.6[f]	−6 to 12%
Dry matter digestibility, %	65.7[f]	69.3[g]	68.9[g]	+5%
Beauchemin *et al.* (1999)				
No. of animals	86	101	—	—
Dry matter intake, Kg/d	10.73	10.62	—	−1%
Weight gain, kg	172[e]	188[d]	—	+9%
Average daily gain, kg/d	1.40[e]	1.53[d]	—	+9%
Feed: Gain	7.72	6.95	—	−11%

[d,e] $P < 0.05$.
[f,g] $P < 0.10$.

Adapted from Beauchemin et al. (2003)

Efficacy of enzymes application to the silage based feeds may be reduced due to presence of certain inhibitory compounds in silage. Nsereko *et al.* (2000) observed that the occurrence of such compounds in barley silage reduces the activity of endo-1,4-β-xylanase obtained from *T. longibrachiatum* up to 23 to 50%, however no effect on was noticed on cellulase activity. Furthermore, addition of exogenous enzymes can accelerate the aerobic deterioration of the silage as they promote the growth of epiphytic microbiota by the release of soluble sugars. This can lead to reduced silage acceptability if time passed between enzyme application and silage offered is very long (Wang *et al.*, 2002). Bowman (2001) investigated the effect an enzyme product (Promote N.E.T., Agribrands International, St. Louis, MO) addition to various proportions of total mixed ration fed to dairy cattle. Enzyme was applied at similar rates to concentrate, supplement or premix portion which were 45, 4 and 0.2% of the total ration. He observed increased NDF digestibility (from 44.3 to 55.6%; 25%) for diet in which enzyme was added to concentrate portion, but other treatments showed no effect on digestibility. When similar diets were tested *in-vitro,* enzymes added to premix portion were also effective. Beauchemin *et al.* (1999) also suggested that the application of exogenous enzymes to larger proportion of the ration increases the chances of their endurance in the rumen. Contrarily, adding the enzymes to a smaller portion of the ration may increase the passage of the enzyme from the fermentation vat, leading to reduced enzyme effects in the rumen. However, passage of enzyme is not an issue in *in-vitro* studies thus batch culture assays may lead towards biased prediction of enzyme effectiveness in the rumen.

Physiological Stage of Animal

Variability in animal responses towards exogenous enzymes application might be related to physiological stage of the animal. Enzymes influence the animal performance in situations in which energy is the first limiting nutrient ion diet or in which digestion of dietary fiber is compromised. High producing animals require higher available energy levels to meet their demands to produce milk or meat and commonly they eat four times more feed than they would eat to fulfill their maintenance requirements. In such feeding conditions, digestibility of fiber may be compromised due to lower ruminal pH and higher passage rates of feed. As compared to maintenance intake levels, each fold increase in feed intake can cause a 4% reduction in nutrient digestibility (NRC, 1989). However, NRC (2001) more recently reported that digestibility

depression at higher feed intake levels of intake can even be greater than formerly estimated. So, at higher level of intakes diets may not be able to be digested potentially. Enzymes have been shown to improve the digestibility of feeds at higher intake levels in producing animals as compared to the situations in which non-producing animals were fed up to their maintenance level (Yang *et al.*, 2000). So, enzymes can be helpful in reducing nutrient losses by helping the animal to attain the potential digestibility of the diets. Similarly, the effect of exogenous enzymes was greater in dairy cows during early lactation than in cows during later stages of lactation (Nussio *et al.*, 1997; Schingoethe *et al.*, 1999). Thus it can be concluded that, direct fed enzymes are more effective when the microbial capability to digest feed is limited by lower ruminal retention times due to higher production and intake levels.

CHAPTER-III

Chemical composition and digestion kinetics of oat grass silage as influenced by varying level of fibrolytic enzymes

Abstract

Experiment was conducted to evaluate the effect of increasing fibrolytic enzyme level on silage characteristics and digestion kinetics of oat grass silage. Fifty day old oat grass was ensiled with 2% molasses and 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme /Kg of dry matter (DM). Oat grass was ensiled in 36 laboratory silos under Completely Randomized Design for 21 days. Dry matter and organic matter (OM) contents remained unaltered (P>0.05) across all treatments. Crude protein (CP), true protein (TP), neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents were affected (P<0.05) by the enzyme treatment. A linear increase in (P<0.05) CP and TP contents was observed with increasing enzyme level. While, its reverse was true for NDF and ADF. Highest NDF and ADF contents were observed in E0, while lowest in E3. Dry matter and OM losses remained unaffected (P>0.05) by any of enzyme level. Crude protein, TP NDF and ADF losses were different (P<0.05) for varying enzyme levels. A linear decrease (P<0.05) in CP and TP losses was noticed in silage treated with increasing enzyme level. Lowest CP and TP losses were observed in E3, which were only 34 and 23% of CP and TP losses observed in E0. In contrast, a linear (P<0.05) increase in NDF and ADF losses was observed with increasing enzyme level. Highest NDF and ADF losses were noted in E3 which were at par with E1 and E2, while lowest in E0 which were only 37 and 36 % of highest NDF and ADF losses. Increasing enzyme level caused a linear decrease (P<0.05) in pH during 1^{st}, 2^{nd} and 3^{rd} week of ensilation. A linear increase (P<0.05) in pH change was also observed with increasing enzyme level during 1st week. Highest pH decrease was observed in E3 which was 56% higher than that observed in E0. However, reverse trend in pH change was noticed during 3rd week of ensilation. Enzyme treatment didn't affect (P>0.05) the extent of digestion and lag time of DM, CP, NDF and ADF for oat silage. Digestibility of CP, NDF and ADF and rate of DM, NDF and ADF digestion also remained unaltered across all treatments. A linear decrease (P<0.05) in DM digestibility was observed with increasing enzyme level. However, rate of CP digestion

increased linearly with increasing enzyme level. On the basis of results, it can be concluded that enzyme application at the time of ensilation can reduce the nutrient losses and fiber contents of silage, without affecting the digestibility of fiber fraction of the silage.

Introduction

In developing countries low per acre yield of fodder and depletion in arable land for fodder production are contributing to decreased nutrient availability by the fodder for livestock (Nisa *et al.*, 2008). Furthermore, scarcity periods during summer and winter worsen the situation (Sarwar *et al.*, 2002). Cutting of fodder at maturity also results in decreased digestibility of the fodder due to increased fiber concentration in plant tissues (Khan et al., 2004), increased lignification (Sarwar et al., 2003; Nisa et al., 2004) and reduced leaf to stem ratio (Hides et al., 1983). This situation advocates the conservation of the fodder as hay and silage to ensure continuous supply of fodder round the year and to preserve the optimum nutrient profile of the fodder.

Ensilation phenomenon is based on natural anaerobic fermentation of fodder in presence of lactic acid producing bacteria, which converts readily fermentable carbohydrates into organic acids (Koc *et al.*, 2008). During this process water soluble carbohydrates are respired and intrinsic plant proteases can convert the protein into ammonia (Muck, 1988). However, early achieved anaerobic conditions and rapid decline in pH can minimize the nutrient losses by reducing respiration and prolonged fermentation (Charmley, 2001). So, a rapid decline in silage pH can improve the fermentation characteristics, nutritive value and utilization of the silage.

Application of fibrolytic enzymes at the time of fodder ensilation may be helpful in achieving rapid pH reduction. They hydrolyze the fiber contents into reducing sugars (Sheperd and Kung 1996a) and thus increase the fermentation rate in silo by providing epiphytic lactic acid producing bacteria with readily fermentable carbohydrates (Stokes, 1992). As a result rate and extent of lactic acid production increases. Higher rates of lactic acid production cause a rapid decline in pH. Also degradation of cell wall contents of the forage by the enzymes may result in higher rate and extent of digestion of silage in the rumen (Bolsen *et al.*, 1995).

So, the present study was planned to examine the nutrient composition and digestion kinetics of oat grass ensiled with escalating level of fibrolytic enzymes.

Materials and Methods

Fifty days old oat grass was procured from the Directorate of Farms, University of Agriculture, Faisalabad. Oat grass was analyzed for DM and was wilted under sun for 3 days to attain 60% moisture.

Preparation of Laboratory silos

After wilting for three days, oat grass was chopped and mixed with mixer. Then mixed oat grass was divided into four heaps of equal size. Each heap was randomly allotted to every treatment. Commercial cellulase+hemicellulase mixture of enzyme (Allzyme®, an *Aspergillus nigar* product by Alltech) was used as an inoculant to ensile oat grass. The enzyme mixture at the rate of 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme /Kg of dry matter (DM) and molasses (2%) were dissolved in water and the solution was sprayed on chopped oat grass at the time of ensilation. Three samples from each heap treated with different level of enzyme were collected to determine silage pH and DM, OM, CP, TP, NDF and ADF contents. Treated oat grass was then ensiled in 36 (3 for each enzyme level) laboratory silos (transparent thick, 40×20 cm polyethylene bags of 2 kg capacity). These silos were pressed for air exclusion and sealed to achieve the anaerobic conditions and were ensiled for 21 days at room temperature (Sarwar *et al.,* 2006). Triplicate silos for each treatment were opened at 7^{th} and 14^{th} day of ensilation to determine the silage pH. After 21 days the remaining silos were opened and samples from each silo was collected and analyzed to determine silage pH, OM, DM, TP, CP, NDF and ADF.

In-situ Trial

Four ruminally cannulated *Nili Ravi* buffalo bulls (400±20 Kg) were used in 4×4 Latin Square Design to evaluate the digestion kinetics of oat grass silage. Nylon bags measuring 10×23 cm with an average pore size of 50 µm were used for the determination of lag time, rate and extent of

disappearance of DM, CP, NDF and ADF for ensiled oat grass silage. For each time point, 10 g sample, in triplicate was poured into bags. Two bags were used to determine DM, CP, NDF and ADF disappearance and the third bag served as blank. The bags were closed and tied with nylon fishing line. Before incubation in the rumen, the bags were soaked in tap water for 15 minutes to remove the sample particles having less than 50 µm size. The weight loss while soaking was recorded as pre-ruminal incubation disappearance. The bags were incubated in the rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 h, in reverse order and were removed all at the same time to reduce variations associated with washing procedure (Sarwar et al., 2004). After removal from the rumen, bags were washed in running tap water until the rinse was clear. The bags were then dried in a forced air oven at 55°C for 48 h. Degradation rates were determined by subtracting the indigestible residue (i.e. the 96 h residence) from the amount in the bag at each time point and then regressing the natural logarithm of that value against time after correcting for lag time (Sarwar et al., 2004). The lag time was calculated according to method described by Mertens and Loften (1980) by using formula.

$$\text{Lag time} = \ln(100) - \text{Intercept} / \text{Rate of digestion}$$

Chemical Analysis

Dry matter of oat silage was determined by drying at 105^{o}C for 4 h followed by equilibration in desiccators (AOAC 1990) and OM was calculated as weight loss upon ignition at 600°C. The CP contents were determined using Kjeldahl method described by AOAC (1990). Neutral detergent fiber and ADF contents were determined with the ANKOM fiber analyzer using reagents described by Van Soest *et al* (1991). For silage pH determination, 10g fresh sample of oat grass was taken just after un-sealing of the silo and was dissolved in 100 mL of distilled water in a 250 mL beaker. Filtrate from water-silage solution was then used to determine pH using a pH-mV meter (HM-21P, TOA Corporation, Tokyo, Japan).

Statistical Analysis

The data collected for each parameter was subjected to analysis of variance technique using multivariate analysis in General Linear Model option of SPSS 17.0 (SPSS Inc., Chicago,

IL, USA). In case of significance (P<0.05) Duncan's New Multiple Range Test was applied to separate the means.

Results

Nutrient composition

After 21 days of ensilation, DM and OM contents remained unaltered (P>0.05) across all treatments. Crude protein was significantly different (P<0.05) across all enzyme levels. A linear increase (P<0.05) in CP contents was observed with increasing enzyme level (Table.3.2). Crude protein was highest in E3 which was at par with E1 and E2, while it was 12.7% higher than that observed in E0. Similar trends was observed for TP. Neutral detergent fiber and ADF contents were also different (P<0.05) for all treatments. A linear decrease (P<0.05) in NDF and ADF contents was observed with increasing enzyme level. Highest NDF and ADF contents were observed in E0, while lowest in E3 (Table.3.2).

Nutrient losses

Dry matter and OM losses remained unaffected (P>0.05) by any of enzyme level. Crude protein and TP losses were different (P<0.05) for varying enzyme levels (Table.3.3). A linear decrease (P<0.05) in CP and TP losses was noticed in silage treated with increasing enzyme level. Lowest CP and TP losses were observed in E3, which were only 34 and 23% of CP and TP losses observed in E0. In contrast, a linear (P<0.05) increase in NDF and ADF losses was observed with increasing enzyme level. Highest NDF and ADF losses were noted in E3 which were at par with E1 and E2, while lowest in E0 which were only 37 and 36 % of highest NDF and ADF losses (Table.3.3).

Silage pH

Silage pH was different (P<0.05) for all treatments during all stages of ensilation (P<0.05). Increasing enzyme level caused a linear decrease (P<0.05) in pH during 1st, 2nd and 3rd week of ensilation. Overall effect of enzyme on pH was also linear. At all stages of ensilation lowest and highest pH was observed in E3 and E0, respectively (Table.3.4). Change in silage pH was different (P<0.05) during 1st and 3rd week and on overall basis (Table.3.5). However, it

remained unaltered (P>0.05) during 2nd week of ensilation. A linear increase (P<0.05) in pH change was observed with increasing enzyme level during 1st week and on overall basis. Highest pH decrease was observed in E3 which was 56% higher than that observed in E0. However, reverse trend in pH change was noticed during 3rd week of ensilation as compared to 1st week (Table.3.5). During 2nd week, change in pH remained unaltered across all treatments (P>0.05).

Digestion kinetics

In-situ digestibility for DM was different (P<0.05) across all treatments (Table.3.6). It was highest in E0 and lowest in E3. However, enzyme treatment didn't affect (P>0.05) the *in-situ* digestibility of CP, NDF and ADF for oat silage (Table.3.7; Table.3.8; Table.3.9.). Extent of digestion for DM, CP, NDF and ADF were also remained unaltered (P>0.05) across all the treatments. Lag time was also similar for all the enzyme levels. Digestion rate was different (P<0.05) for CP across different treatments (Table.3.7) and was highest in E2 and lowest in E0. However, digestion rate for DM, NDF and ADF remained unaltered (P>0.05). A linear (P<0.05) decrease in digestible and potentially digestible fraction was observed for DM, NDF and ADF with increasing enzyme level. While, digestible and potentially digestible fraction of CP showed a linear increase (P<0.05) with increasing enzyme level.

Table.3.1 Nutrient composition of oat grass at ensilation

Items	g/ Kg
Dry matter	301±6.63
Organic matter	930±9.26
Crude protein	132±5.56
True protein	112±3.47
Neutral detergent fiber	397±5.7
Acid detergent fiber	223±5.17

Table.3.2 Nutrient composition of oat grass silage after 21 days of ensilation

Items (g/Kg)	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
Dry matter	288	286	288	289	1.1	NS	NS
Organic matter	902	896	892	900	2.7	NS	NS
Crude protein	114.5^{b}	127.2^{a}	128.7^{a}	129^{a}	1.6	*	NS
True protein	98.4^{b}	105^{a}	107.2^{a}	108.6^{a}	0.7	*	NS
Neutral detergent fiber	368^{a}	343^{b}	330.7^{bc}	324^{c}	2	*	NS
Acid detergent fiber	208.3^{a}	185.3^{b}	186.3^{b}	181.7^{b}	2.5	*	NS

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Table.3.3 Effect of increasing level of enzyme application on nutrient losses in oat grass silage during ensilation

Items (g/Kg)	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
Dry matter	13.3	15	13	12	1.1	NS	NS
Organic matter	27.8	33.6	37.5	29.8	2.7	NS	NS
Crude protein	17.5^{a}	4.7^{b}	3.3^{b}	2.9^{b}	1.6	*	NS
True protein	13.5^{a}	6.9^{b}	4.7^{b}	3.3^{b}	0.7	*	NS
Neutral detergent fiber	28.5^{c}	52.8^{b}	65.8^{ab}	72.5^{a}	2.0	*	NS
Acid detergent fiber	14.5^{b}	36.5^{a}	37.5^{a}	42.2^{a}	2.5	*	*

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Table.3.4 Effect of increasing level of enzyme application on oat grass silage pH

pH	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
0 day	6.65	6.64	6.63	6.66	0.02	NS	NS
1st week	4.95[a]	4.56[b]	4.53[b]	4.49[b]	0.025	*	*
2nd week	4.87[a]	4.53[b]	4.48[b]	4.44[b]	0.03	*	*
3rd week	4.68[a]	4.44[b]	4.39[b]	4.36[b]	0.028	*	NS
Overall[3]	4.83[a]	4.51[b]	4.46[b]	4.43[b]	0.026	*	*

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

[3]Averaged pH at 1st, 2nd and 3rd week of ensilation.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Table.3.5 Effect of increasing level of enzyme application on change in oat grass silage pH

pH change	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
1st week	-1.4[b]	-2.08[a]	-2.1[a]	-2.16[a]	0.02	*	NS
2nd week	-0.15	-0.03	-0.05	-0.05	0.028	NS	NS
3rd week	-0.47	-0.27	-0.24	-0.22	0.039	*	NS
Overall[3]	-1.66[b]	-2.19[a]	-2.21[a]	-2.27[a]	0.053	*	NS

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

[3]Averaged pH at 1st, 2nd and 3rd week of ensilation.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Table.3.6 Effect of increasing level of enzyme application on dry matter digestion kinetics of oat grass silage

Items	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
Digestibility[3] (%)	79.5[a]	78.25[ab]	76.4[c]	77.45[bc]	0.21	*	NS
Digestible fraction[4]	228.9[a]	223.7[b]	220[b]	223.8[b]	0.60	*	*
Extent of digestion[3] (%)	80.5	80.5	78.8	80.2	0.40	NS	NS
Potentially digestible fraction[4]	231.8	230.2	226.8	231.9	1.27	NS	NS
Lag time (h)	1.45	1.44	1.42	1.45	0.01	NS	NS
Digestion rate (%/ h)	5.22	5.27	5.26	5.23	0.48	NS	NS

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

[3] Digestibility and extent of digestion were determined after 48 and 96 hours of ruminal incubation, respectively.

[4] Fraction (g/Kg dry matter) remaining at 0 h of incubation.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Table.3.7 Effect of increasing level of enzyme application on crude protein digestion kinetics of oat grass silage

Items	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
Digestibility[3] (%)	89.1	88.6	89.3	89.8	0.28	NS	NS
Digestible fraction[4]	102^{c}	112.7^{b}	115.6^{a}	115.3^{ab}	0.36	*	*
Extent of digestion[3] (%)	92.7	93.0	92.9	93.1	0.21	NS	NS
Potentially digestible fraction[4]	106.2^{b}	118.3^{a}	119.6^{a}	120.1^{a}	0.27	*	*
Lag time (h)	0.89	0.91	0.90	0.90	0.01	NS	NS
Digestion rate (%/ h)	5.08^{b}	5.08^{b}	5.21^{a}	5.15^{ab}	0.01	*	NS

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

[3] Digestibility and extent of digestion were determined after 48 and 96 hours of ruminal incubation, respectively.

[4] Fraction (g/Kg dry matter) remaining at 0 h of incubation.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Table.3.8 Effect of increasing level of enzyme application on neutral detergent fiber digestion kinetics of oat grass silage

Items	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
Digestibility[3] (%)	53.6	53.2	53.1	53.8	0.25	NS	NS
Digestible fraction[4]	197.4^{a}	182.5^{b}	175.6^{c}	174.2^{c}	0.86	*	*
Extent of digestion[3] (%)	61.3	60.4	60.2	60.3	0.3	NS	NS
Potentially digestible fraction[4]	225.6^{a}	207.1^{b}	199.1^{bc}	195.4^{c}	1.0	*	*
Lag time (h)	1.94	1.93	1.92	1.94	0.01	NS	NS
Digestion rate (%/ h)	4.75	4.77	4.83	4.95	0.28	NS	NS

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

[3] Digestibility and extent of digestion were determined after 48 and 96 hours of ruminal incubation, respectively.

[4] Fraction (g/Kg dry matter) remaining at 0 h of incubation.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Table.3.9 Effect of increasing level of enzyme application on acid detergent fiber digestion kinetics of oat grass silage

Items	Treatments[1]				SE	Probabilities[2]	
	E0	E1	E2	E3		L	Q
Digestibility[3] (%)	43.6	45	45.4	46.3	0.4	NS	NS
Digestible fraction[4]	90.7[a]	83.5[b]	84.7[b]	83[b]	0.7	*	NS
Extent of digestion[3] (%)	53.1	52.7	52.2	53.1	0.2	NS	NS
Potentially digestible fraction[4]	110.6[a]	97.6[b]	97.3[b]	96.5[b]	0.5	*	*
Lag time (h)	2.13	2.10	2.05	2.07	0.03	NS	NS
Digestion rate (%/ h)	4.59	4.70	4.60	4.70	0.03	NS	NS

[1]E0, E1, E2 and E3 represent oat grass ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2] L= Linear and Q= quadratic responses towards increasing enzyme level.

[3] Digestibility and extent of digestion were determined after 48 and 96 hours of ruminal incubation, respectively.

[4] Fraction (g/Kg dry matter) remaining at 0 h of incubation.

NS= Non-significant (P>0.05) and *= significant (P<0.05).

SE= Standard error.

[a,b,c] Means sharing different superscripts differ significantly (P<0.05).

Discussion

Nutrient composition

Unaltered DM and OM contents across all treatments at 21 days of ensilation were supported by the findings of other researchers (Kung *et al.*, 1990; Zahiroddini *et al.*, 2004) who reported similar DM and OM for cellulase treated barley silage. Similar DM and OM for oat silage observed in the present study might be related to similar losses of DM and OM in all silages. Improved CP and TP contents in enzyme treated silages were supported by the findings of Rodrigues *et al.* (2001). Linear increase in CP and TP in response to increasing enzyme level might be due to a linear decrease in pH which might have reduced the proteolytic activity in silage (Muck, 1988). Decreasing trend in NDF and ADF contents in response to increasing enzyme level were in line with the findings of Adogla-Bessaa *et al.* (1999). Reduction in the fiber contents of the silage is a major effect of enzyme addition (Stokes and Chen, 1994). Sheperd and Kung (1996b) and Nadeau *et al.* (2000) also reported decreased fiber contents of silage treated with cellulase. Reduction in fiber contents in response to enzyme treatment might be related to conversion of some fiber fraction to the reducing sugars by the enzymes (Kung *et al.*, 2003). Sheperd and Kung (1996a) observed a linear increase in glucose contents of maize silage collected at three different stages of age in response to increasing levels of Cornzyme® application. Linear increase in fermentable carbohydrates in response to enzyme levels may explain the linear decrease in fiber fractions. However, Mandebvu *et al.* (1999) reproted that addition of fibrolytic enzymes to the Bermuda grass silage had no effect on cell wall composition and end products of silage fermentation. This might be related to forage specific nature of the enzymes (Jalilvand *et al.*, 2008).

Nutrient losses

Dry matter and OM losses remained unaffected by the enzyme application. Adogla-Bessaa *et al.* (1999) also reported similar results. However, results of the present study were not supported by the findings of other researchers, who reported lower (Dean *et al.*, 2005; Adogla-Bessa and Owen, 1995; Stokes and Chen 1994) or higher (Stokes and Chen, 1994) DM losses in response to cell wall degrading enzyme application. A linear decrease in CP and TP losses might be related to a linear decrease in pH. Change in pH was also more rapid for enzyme treated silage in present study, which by inhibiting prolonged fermentation

(Muck, 1993), might have decreased the conversion of protein into ammonia (Kung, 2000; Keady and Murphy, 1997). Reduced CP and TP losses were supported by the findings of Sun *et al.* (2009) and Xing *et al.* (2009). Higher NDF and ADF losses observed in present study are inconsistent with the findings of other researchers (Xing *et al.*, 2009; Adogla-Bessaa *et al.*, 1999) who reported higher NDF and ADF losses for enzyme treated silages as compared to the un-treated silage. Higher NDF and ADF losses might be related degradation of cell wall contents by the enzyme action and subsequent release of sugars (Sheperd and Kung 1996a).

Silage pH

In present study change in silage pH was more rapid in enzyme treated silages during 1st week. Rapid decline in silage pH can prevent the plant protein from degradation and mold and undesirable microbial species growth (Kung, 2000). So, the enzyme treated silages showed lower CP and TP losses in present study. Decrease in pH in response to enzyme application was in concordance with findings of other researchers (Sun *et al.*, 2009; Dean *et al.*, 2005; Colombatto *et al.*, 2004; Adogla-Bessa *et al.*; 1999), who reported lower pH for enzyme treated silages as compared to the untreated silage. Reduction in pH in response to enzyme treatment was might be due to availability of fermentable carbohydrates and increased growth of epiphytic bacteria (Kung *et al.*, 2003); this release of fermentable sugars might have increased the rate and extent of lactic acid production in the silage (Kozelov *et al.*, 2008). So, higher lactic acid production in enzyme treated silage might have resulted in declined pH (Spoelstra *et al.*, 1992). However, Zahiroddini *et al.* (2004) reported unchanged pH and lactic acid contents for whole crop barley silage treated with SilagePro® as compared to the untreated silage. Higher pH change in untreated silage during 3rd week of ensilation showed that enzymes cause maximum reduction during initial phases of ensilation.

Digestion kinetics

Unaltered CP, NDF and ADF digestibility observed in present study was in line with findings of other researchers (Kozelov *et al.*, 2008; Nadeau *et al.*, 2000; Mandebvu *et al.*, 1999). However, results of present study are inconsistent with findings of Xing *et al.* (2009) and Colombatto *et al.* (2004) who reported improved NDF digestibility for silages treated with enzyme as compared to the untreated silage. Decreased DM digestibility in enzyme treated silage was might be due to less digestible DM remaining in the silage after hydrolysis

of fiber by enzyme during ensiling (Nadeau *et al.*, 1996). So, a linear decrease in digestible and potentially digestible nutrient fractions might have reduced the effect of enzyme application on digestibility of NDF and ADF.

Unaltered digestion rates and lag time for DM, NDF and ADF were supported by the findings of Zhu *et al.* (2011) and Zahiroddini *et al.* (2004). However, enzyme application should affect lag time as they release sugars from the fiber which help the microbes to get attached to their substrate through chemotaxis (Newbold, 1997). But released sugars from the fiber, might have utilized by the lactic acid producing bacteria in silo (Kung *et al.*, 2003), which explain the reason that why did enzymes have no effect on digestion kinetics. Higher digestion rates for CP observed in enzyme treated silage was might be due to higher digestible fraction of CP in enzyme treated silages.

Conclusions

Application of fibrolytic enzyme at the time of ensilation can reduce the nutrient losses and fiber contents of oat grass silage and reducing effect increases with increasing the rate of application. However, escalating enzyme level linearly reduces the digestible fractions of NDF and ADF, but has less effect on digestion kinetics of oat grass silage.

CHAPTER-IV

Effect of varying concentration of fibrolytic enzymes on chemical composition and digestion kinetics of urea-molasses treated wheat straw

Abstract

Experiment was conducted to evaluate the effect of increasing fibrolytic enzyme level on nutrient composition and digestion kinetics of urea treated wheat straw. Wheat straw (WS) was treated with 4% urea and 6% molasses and was ensiled with 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme /Kg of dry matter (DM). Enzyme mixture was dissolved in water and the solution was sprayed on WS. Then after an hour of enzyme treatment, molasses and urea were dissolved in water and sprayed on enzyme-treated WS. Wheat straw was ensiled in 36 laboratory silos under Completely Randomized Design for twenty one days. Application of enzymes at the time of ensilation of WS didn't affect ($P>0.05$) the DM, crude protein, true protein, neutral detergent fiber (NDF) and acid detergent fiber (ADF) contents. Changes in these nutrients during ensilation were also remained unaltered ($P>0.05$). Overall pH of WS ensiled with varying enzyme level ranged from 8.42 to 8.47. Enzyme treatment didn't affect ($P>0.05$) the pH of the ensiled WS. Lag time, digestion rate, *in-situ* digestibility and extent of digestion of DM, NDF and ADF also remained unaltered ($P>0.05$) across all the treatments. On the basis of results it is concluded that enzyme did not affect the nutrient profile of WS because of alkaline pH due to rapid production of ammonia in the silo. However, further research regarding the ensilation of WS with varying urea, molasses, organic acids and lactic acid producing bacteria may be helpful in improving the efficacy of enzymes by reducing the pH of the silo.

Introduction

In developing countries, components of ruminant feeds mainly comprise of crop residues (Sarwar et al., 2002). Among crop residues, WS is abundantly available by-product of wheat crop production. However, its low protein, high fiber contents and low digestibility limit its use in ruminant nutrition (Abo-Eid *et al.*, 2007). Nutritive value of WS can be improved by the application of various physical, biological and chemical treatments (Sarwar, et al., 2002). Among chemical treatments, ammoniation of WS by using urea is most commonly adopted technique to increase the nitrogen contents of the WS (Sarwar et al., 2006). However, ammonia losses during ensilation of urea treated reduce the efficiency of the treatment (Khan *et al.*, 2007). Better fixation of ammonia can be achieved by the addition of molasses (Sarwar et al., 2006) and by maintaining lower pH in the silo (Khan et al., 2006). Lower silo pH can be maintained by using certain additives like fibrolytic enzymes.

Fibrolytic enzymes used in animal feeds include cellulases, hemicellulases, pectinases etc. They are the products of batch fermentation (Cowan, 1994) and usually are of bacterial and fungal origin (Pendleton, 2000). Application of fibrolytic enzymes at the time of ensilation results in conversion of fiber contents of the forage into fermentable sugars which are then used by the microbes to produce lactic acid (McDonald *et al.*, 1991). They have been reported to reduce the fiber contents and pH of grass (Rodrigues *et al.*, 2001; Selmer-Olsen *et al.*, 1993), legume (Nadeau and Buxton, 1997), whole plant (Zahiroddini *et al.*, 2004; Adogla-Bessa *et al.*, 1999) and maize stover silage (Sun et al., 2009). They have also been reported to improve the digestibility of rice straw (Liu and Ørskov, 2000). However, studies regarding the effect of fibrolytic enzyme application at the time of ensilation of urea-treated WS on the pH and fiber contents of the ensiled WS are limited.

Ensilation of urea-treated WS with fiber degrading enzymes may result in improved ammonia fixation and reduced fiber contents of the ensiled WS. Keeping in view, the study was planned to examine the nutrient composition and digestion kinetics of urea-treated WS ensiled with varying level of fibrolytic enzymes.

Materials and Methods

Wheat straw was procured from the Directorate of Farms, University of Agriculture, Faisalabad, and was ground through a Wiley mill (2 mm screen) for chemical analyses.

Preparation of Laboratory silos

Commercial cellulase+hemicellulase mixture of enzymes (Allzyme®, an *Aspergillus nigar* product by Alltech) was used as an inoculant to ensile WS. Wheat straw (WS) was treated with 4% urea and 6% molasses and was ensiled with 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme

/Kg of dry matter (DM). Enzyme mixture was dissolved in water and was sprayed on WS. Then after an hour of enzyme treatment, molasses and urea was dissolved in water and the solution was sprayed on enzyme-treated WS. Amount of water used was sufficient to attain 50% moisture in WS. The treated WS was then ensiled in 36 (3 for each enzyme level) laboratory silos (transparent thick, 40×20 cm polyethylene bags of 2 Kg capacity). These silos were pressed for air exclusion and sealed to achieve the anaerobic conditions and were ensiled for 21 days at room temperature as reported by Sarwar *et al.* (2006). Triplicate silos for each treatment were opened at 7^{th} and 14^{th} day of ensilation to determine the pH of ensiled WS. After 21 days the remaining silos were opened and sample from each silo were collected and analyzed to determine pH and OM, DM, TP, CP, NDF and ADF contents of ensiled WS.

In-situ Trial

Four ruminally cannulated *Nili Ravi* buffalo bulls (400±20 Kg) were used in 4×4 Latin Square Design to evaluate the digestion kinetics of ensiled WS. Nylon bags measuring 10×23 cm with an average pore size of 50 µm were used for the determination of lag time, rate and extent of disappearance of DM, CP, NDF and ADF for ensiled WS. For each time point, 10 g sample, in triplicate was poured into bags. Two bags were used to determine DM, CP, NDF and ADF disappearance and the third bag served as blank. The bags were closed and tied with nylon fishing line. Before incubation in the rumen, the bags were soaked in tap water for 15 minutes to remove the sample particles having less than 50 µm size. The weight loss while soaking was recorded as pre-ruminal incubation disappearance. The bags were incubated in the rumen for 0, 1, 2, 4, 6, 10, 16, 24, 36, 48 and 96 h, in reverse order and were removed all at the same time to reduce variations associated with washing procedure (Sarwar et al., 2004). After removal from the rumen, bags were washed in running tap water until the rinse was clear. The bags were then dried in a forced air oven at 55°C for 48 h. Degradation rates were determined by subtracting the indigestible residue (i.e. the 96 h residence) from the amount in the bag at each time point and then regressing the natural logarithm of that value against time after correcting for lag time (Sarwar et al., 2004). The lag time was calculated according to method described by Mertens and Loften (1980) by using formula.

$$\text{Lag time} = \ln(100) - \text{Intercept} / \text{Rate of digestion}$$

Chemical Analysis

The DM of WS was determined by drying at 105°C for 4 h followed by equilibration in desiccators (AOAC 1990) and OM was calculated as weight loss upon ignition at 600°C. The CP contents were determined using Kjeldahl method described by AOAC (1990).

Neutral detergent fiber and ADF contents were determined with the ANKOM fiber analyzer using reagents described by Van Soest *et al* (1991). Ensiled WS pH was determined by using a pH-mV meter (HM-21P, TOA Corporation, Tokyo, Japan).

Statistical Analysis

The data collected for each parameter was subjected to analysis of variance technique using multivariate analysis in General Linear Model option of SPSS 17.0 (SPSS Inc., Chicago, IL, USA). In case of significance ($P<0.05$) Duncan's New Multiple Range Test was applied to separate the means.

Table.4.1 Nutrient composition of urea-treated wheat straw at ensilation

Items	g/ Kg
Dry matter	502.2±7.8
Organic matter	915.8±8.7
Crude protein	113.8±5.1
True protein	33.2±1.8
Neutral detergent fiber	785±8.2
Acid detergent fiber	506±8.1

Results

Nutrient composition

Dry matter, CP, TP, NDF and ADF were not influenced (P>0.05) by varying enzyme levels (Table.4.2). Similarly, changes in these nutrients after 21 days of ensilation was also similar (P>0.05) for all enzyme levels (Table.4.3).

pH

Unaltered pH (P>0.05) was observed for all enzyme levels at all weeks of ensilation (Chart 4.1; 4.2; 4.3). Similarly, increasing enzyme level didn't affect (P>0.05) overall pH of ensiled wheat straw. On overall basis, pH of the silos ranged from 8.42 to 8.47 (Chart.4.4).

Digestion kinetics

Increasing enzyme level didn't influence (P>0.05) *in-situ* digestibility, extent of digestion, lag time and digestion rate of DM, CP, NDF and ADF (Table 4.4; 4.5).

Table.4.2 Nutrient composition of ensiled wheat straw after 21 days of ensilation

Items (g/ Kg)	Treatments[1]				SE
	E0	E1	E2	E3	
Dry matter	498.3	495.7	498	497.3	3.6
Organic matter	890.6	894.6	891.7	889.6	3.7
Crude protein	90.4	89	90.4	90.4	0.7
True protein	45.2	45.6	46.7	47.4	0.9
Neutral detergent fiber	755.7	755	751.3	748.3	2.4
Acid detergent fiber	488.3	493	493.7	487.3	2.4

[1]E0, E1, E2 and E3 represent urea-treated wheat straw ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

SE= Standard error.

Table.4.3 Effect of increasing level of enzyme application on changes in nutrient profile of ensiled wheat straw

Items (g/ Kg)	Treatments[1]				SE
	E0	E1	E2	E3	
Dry matter	-3.9	-6.5	-4.2	-4.9	3.6
Organic matter	-25.1	-21.1	-24	-26.1	3.7
Crude protein	-23.4	-23.4	-23.4	-24.8	0.7
True protein	12	12.4	13.5	14.2	0.9
Neutral detergent fiber	-29.3	-30	-33.7	-36.7	2.4
Acid detergent fiber	-17.7	-13	-12.3	-18.7	2.4

[1]E0, E1, E2 and E3 represent urea-treated wheat straw ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

SE= Standard error.

Chart. 4.1 pH of ensiled wheat straw at 1st week of ensilation

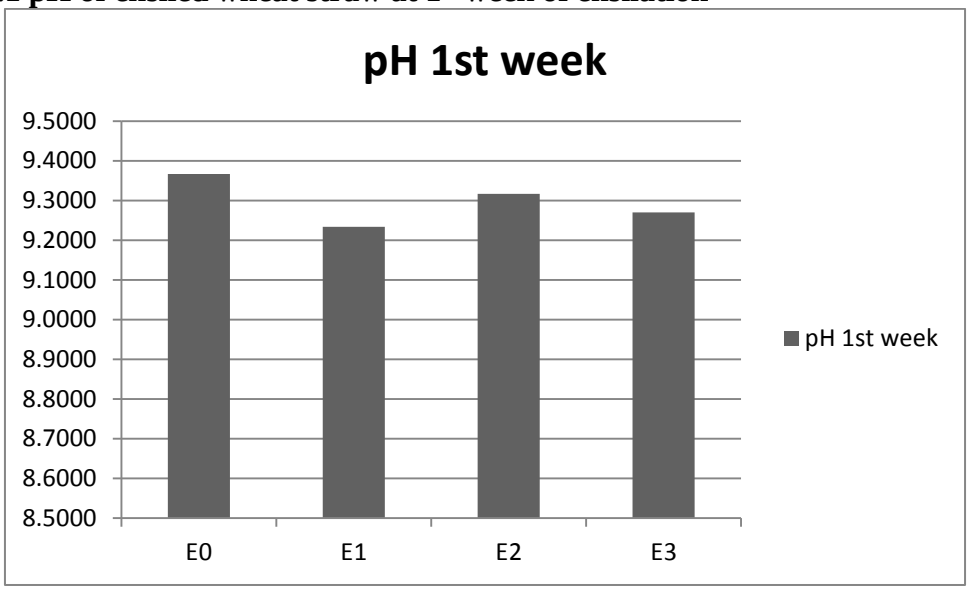
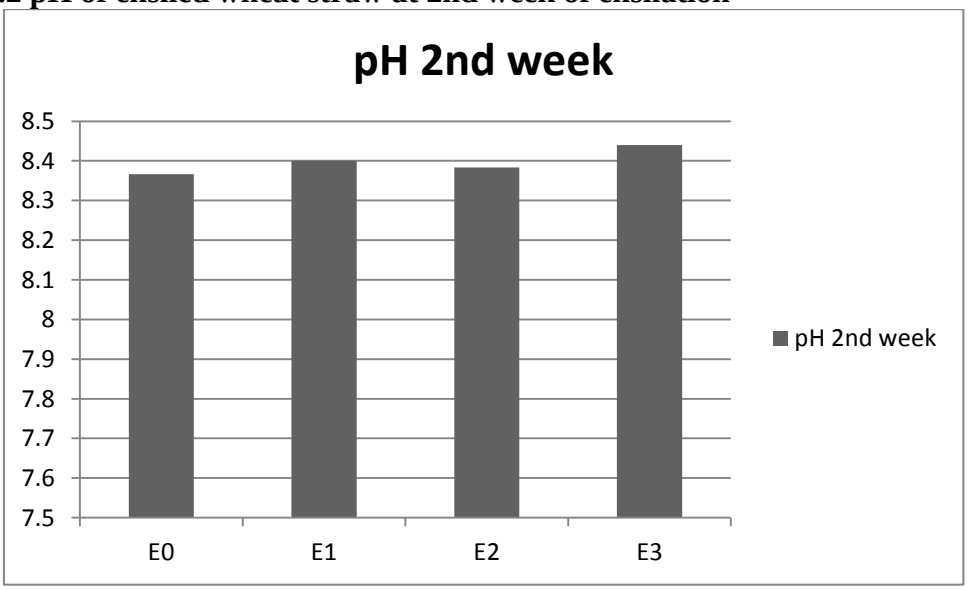


Chart. 4.2 pH of ensiled wheat straw at 2nd week of ensilation



E0, E1, E2 and E3 represent urea-treated wheat straw ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

Chart. 4.3 pH of ensiled wheat straw at 3rd week of ensilation

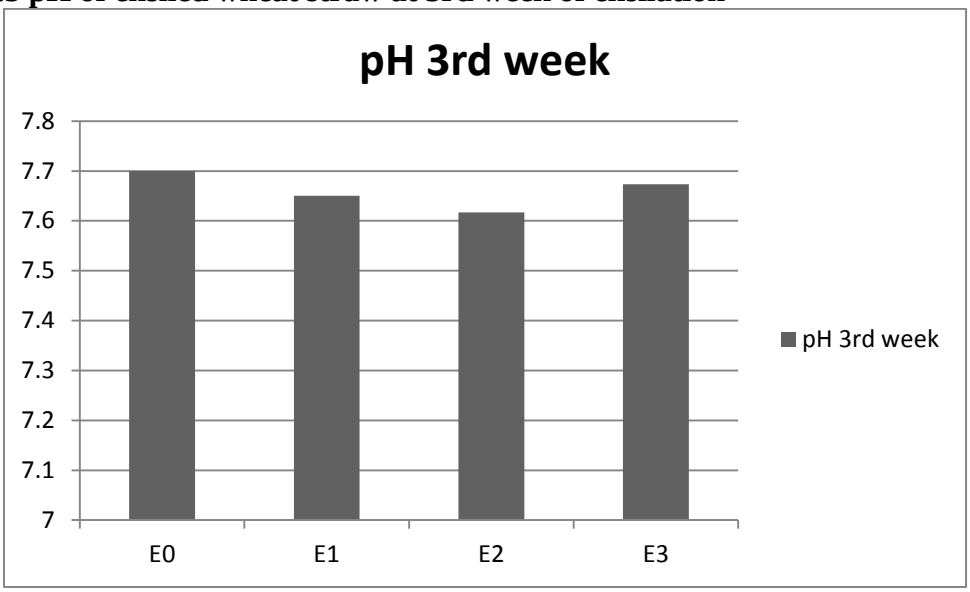
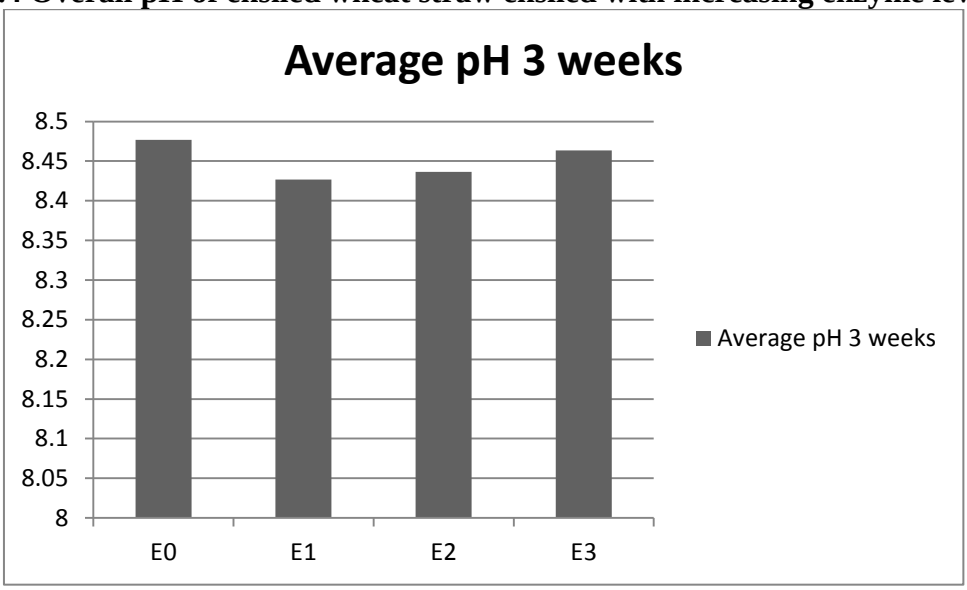


Chart. 4.4 Overall pH of ensiled wheat straw ensiled with increasing enzyme levels



E0, E1, E2 and E3 represent urea-treated wheat straw ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

Table.4.4 Effect of increasing level of enzyme application on dry matter and crude protein digestion kinetics of ensiled wheat straw

Items	Treatments[1]				SE
	E0	E1	E2	E3	
Dry matter					
Digestibility[2] (%)	52.1	52.3	51.5	51.8	0.41
Extent of digestion[2] (%)	62.7	61.9	61.6	62.5	0.56
Lag time (h)	3.16	3.17	3.16	3.15	0.01
Digestion rate (%/h)	4.7	4.74	4.72	4.75	<0.001
Crude protein					
Digestibility[2] (%)	56.1	55	55.5	56.4	0.41
Extent of digestion[2] (%)	64	64.5	64.9	65	0.29
Lag time (h)	0.76	0.77	0.76	0.77	0.009
Digestion rate (%/h)	4.67	4.65	4.63	4.67	<0.001

[1]E0, E1, E2 and E3 represent urea-treated wheat straw ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2]Digestibility and extent of digestion were determined after 48 and 96 hours of ruminal incubation, respectively.

SE= Standard error.

Table.4.5 Effect of increasing level of enzyme application on neutral detergent fiber and acid detergent fiber digestion kinetics of ensiled wheat straw

Items	Treatments[1]				SE
	E0	E1	E2	E3	

Neutral detergent fiber					
Digestibility[2] (%)	48.5	48.1	48.2	49.1	0.31
Extent of digestion[2] (%)	59.2	59.1	60.1	59.6	0.34
Lag time (h)	3.24	3.24	3.25	3.27	0.02
Digestion rate (%/h)	4.56	4.62	4.59	4.61	<0.001
Acid detergent fiber					
Digestibility[2] (%)	43.6	42.8	43.4	43.8	0.55
Extent of digestion[2] (%)	53.2	53	52.7	53.2	0.46
Lag time (h)	3.77	3.74	3.76	3.76	0.01
Digestion rate (%/h)	4.62	4.67	4.7	4.67	<0.001

[1]E0, E1, E2 and E3 represent urea-treated wheat straw ensiled with enzyme at the rate of 0, 1, 2 and 3g/Kg of dry matter, respectively.

[2]Digestibility and extent of digestion were determined after 48 and 96 hours of ruminal incubation, respectively.

SE= Standard error.

Discussion

Nutrient composition

Unaltered DM, CP, TP, NDF and ADF contents of ensiled WS might be related to similar rate and extent of fermentation in all silos which reflected in similar nutrient changes for all treatments. Adolga-Bessa et al. (1999) also reported similar findings for urea-treated whole crop wheat ensiled with enzyme mixture. But in previous experiment increasing enzyme level linearly increased CP and TP and reduced NDF and ADF contents of oat silage. Inconsistent results might be related to forage specific nature of the enzyme. Jalilvand *et al.* (2008) concluded that responses to the enzyme addition differ with the forage type and the activity of enzyme. Furthermore, enzymes have more effect on immature plants as compared to mature plants with crystalline fiber structure (Van Vuuren et al., 1989). Lignin structure and phenolic interactions with polysaccharides might have limited the enzyme action on cell wall components of WS (Chesson, 1993). Another plausible reason may be the rapid production of ammonia due to urea treatment of WS (Sarwar et al., 2003) which reflected in alkaline pH of silos throughout the ensilation phase. This higher pH might have restrained the enzyme activity (Vicini et al., 2003) as enzyme used in present study was of fungal origin and most the fungal enzymes work optimally at pH from 4-6 (Gashe, 1992; Muzakhar et al., 1998).

pH

Unaltered pH of urea-treated WS in response to enzyme treatment is supported by the findings of Adolga-Bessa et al. (1999) who also reported similar pH of urea-treated whole crop wheat ensiled with increasing level of cell wall degrading enzyme. During 21 days of ensilation, pH of all silos remained alkaline and it ranged from 8.42 to 8.47 on overall basis. Alkaline pH might be related to rapid production of ammonia in the silo (Sarwar et al., 2003). This higher production of ammonia might have resulted in lower activity of enzymes which ultimately resulted in reduced conversion of fiber into reducing sugars. By the utilization of sugars epiphytic bacteria produce lactic acid (Kung *et al.*, 2003); so similar availability of fermentable sugars in all silos might have resulted in similar rate and extent of lactic acid production (Kozelov *et al.*, 2008) and thus unaltered pH.

Digestion kinetics

Unaltered DM, CP, NDF and ADF digestibility and rate and extent of digestion are not supported by the findings of Hussain (2009) who reported higher *in-situ* digestibility, extent and rate of digestion and lower lag time for DM and NDF of Berseem+ WS diet treated with exogenous enzyme mixture. Enzymes convert the fiber into reducing sugars

which help the microbes to get attached to their substrate through chemotaxis (Newbold, 1997) resulting in higher digestibility. Rodriguez et al., (2007) stated that application of fibrolytic enzymes increases the soluble fraction of the DM which can lead to higher digestibility. But the production of reducing sugars is dependent upon fiber composition of the forage. Nadeau et al. (2000) reported lower production of reducing sugars for forage with higher lignin contents in response to enzyme treatment. Thus no effect of enzyme application on digestion kinetics of WS might be related to inability of enzymes to hydrolyze the complex structure of the WS fiber.

Conclusions

Enzyme treatment at the time of ensilation of urea-treated WS has no effect on its chemical composition and digestion kinetics. Further experiments using varying level of urea with organic acids and fibrolytic enzymes to ensile WS should be carried out.

CHAPTER-V

Summary

Two independent experiments were conducted to evaluate the chemical composition and digestion kinetics of oat grass silage and urea treated wheat straw (WS) as influenced by varying level of exogenous fibrolytic enzymes. In experiment-I, fifty day old oat grass was ensiled with 2% molasses and 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme /Kg of dry matter (DM). Oat grass was ensiled in 36 laboratory silos under Completely Randomized Design for 21 days. At 7th and 14th day of ensilation, triplicate silos for each treatment were opened and silage pH was determined. After 21 days the remaining silos were opened and samples were analyzed for pH, DM, organic matter (OM), crude protein (CP), true protein (TP), neutral detergent fiber (NDF) and acid detergent fiber (ADF). Then four ruminally cannulated *Nili Ravi* buffalo bulls were used in 4×4 Latin Square Design to determine *in-situ* DM, CP, NDF and ADF disappearance, lag time and rate and extent of digestion of oat grass silage. In experiment-II WS was treated with 4% urea and 6% molasses and was ensiled with 0 (E0), 1 (E1), 2 (E2) and 3 (E3) g of enzyme /Kg of DM. Enzyme mixture was dissolved in water and the solution was sprayed on WS. Then after an hour of enzyme treatment, molasses and urea were dissolved in water and sprayed on enzyme-treated WS. Rest of the experiment was conducted following same procedure as mentioned for oat grass silage.

In experiment-I, DM and OM contents remained unaltered ($P>0.05$) across all treatments. Crude protein, TP, NDF and ADF contents were affected ($P<0.05$) by the enzyme treatment. A linear increase ($P<0.05$) in CP and TP contents was observed with increasing enzyme level. However, a linear decrease ($P<0.05$) in NDF and ADF contents was observed with increasing enzyme level. Highest NDF and ADF contents were observed in E0, while lowest in E3 enzyme level. Dry matter and OM losses remained unaffected ($P>0.05$) by any of enzyme level. Crude protein, TP NDF and ADF losses were different ($P<0.05$) for varying enzyme levels. A linear decrease ($P<0.05$) in CP and TP losses was noticed in silage treated with increasing enzyme level. Lowest CP and TP losses were observed in E3, which were only 34 and 23% of CP and TP losses observed in E0. In contrast, a linear increase ($P<0.05$) in NDF and ADF losses was observed with increasing enzyme level. Highest NDF and ADF losses were noted in E3 which were at par with E1 and E2, while lowest in E0 which were only 37 and 36 % of highest NDF and ADF losses. Increasing enzyme level caused a linear

decrease ($P<0.05$) in pH during 1st, 2nd and 3rd week of ensilation. A linear increase ($P<0.05$) in pH change was also observed with increasing enzyme level during 1st week. Highest pH decrease was observed in E3 which was 56% higher than that observed in E0. However, reverse trend in pH change was noticed during 3rd week of ensilation. Enzyme treatment didn't affect ($P>0.05$) the extent of digestion and lag time of DM, CP, NDF and ADF for oat grass silage. Digestibility of CP, NDF and ADF and rate of DM, NDF and ADF digestion also remained unaltered across all the treatments. A linear decrease ($P<0.05$) in DM digestibility was observed with increasing enzyme level. However, rate of CP digestion increased linearly ($P<0.05$) with increasing enzyme level.

In experiment-II, application of enzymes at the time of ensilation of WS didn't affect ($P>0.05$) the DM, CP, TP, NDF and ADF contents. Changes in these nutrients during ensilation were also remained unaltered ($P>0.05$). Overall pH of WS ensiled with varying enzyme level ranged from 8.42 to 8.47. Enzyme treatment didn't affect ($P>0.05$) the pH of the ensiled WS. Lag time, digestion rate, *in-situ* digestibility and extent of digestion of DM, NDF and ADF also remained unaltered ($P>0.05$) across all the treatments. Unaltered results might be related to rapid production of ammonia in the silo, which resulted in alkaline pH and reduced enzyme activity.

Findings of the study indicate that the enzyme application at the time of ensilation can reduce the nutrient losses and fiber fractions of oat grass silage, without affecting the digestibility of fiber fraction of the silage. However, enzyme application has no effect on chemical composition and digestion kinetics of urea-treated WS.

CHAPTER-VI

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