EFFECT OF PREDIGESTED LOCAL FORAGES WITH EXOGENOUS FIBROLYTIC ENZYMES ON CHEMICAL COMPOSITION AND IN-VITRO DIGESTIBILITY.

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SUMMARY

This study was implemented to evaluate the effects of predigestion for some local forages: alfalfa hay (AH), barley straw (BS), reed (R) and combination of these forages to enhance nutritive value by predigestion to the cell wall with different commercial fibrolytic enzyme powder levels at 0, 1, 2, and 3% by socking it for 12h. Results indicated high significant increases (P<0.01) in dry matter content (DM) and inorganic matter (Ash) for predigestion to all forages with enzyme, wile they were high significant reduce (P<0.01) in organic matter (OM) and neutral detergent fiber (NDF) for all treated forages with enzyme, but not affected on CP and ADL content. In vitro trial indicated increase in dry matter digestibility (DMD) and organic matter digestibility (OMD) (P<0.01) for predigested alfalfa hay (AH) and not significant increases for the other forages with sheep rumen liquor. When used goat rumen liquor, OMD for alfalfa hay was significant increases (P<0.01). In conclusion: Alfalfa hay had the best values for in vitro DMD and OMD. Wild reed (R) had the lowest values. The level of enzyme was not significant and the best improvement with the level 3% on in vitro DMD and OMD. There was no significant differ for in vitro DMD and OMD when used sheep or goat rumen fluid.

Keywords: *Alfalfa hay, barley straw, reed hay, in vitro digestibility.*

INTRODUCTION

Forages are the major and cheapest food for ruminants, improvement in forage digestion increases the energy available to ruminants and the nutrient availability for better utilization of these forages. In this direction, numerous methods have been tried to upgrade the nutritive value of low quality forages such as physical, chemical, and biological treatment or use feed additives. (Hassan *et al.*, 1998; Hassan and Tawffek, 2009; Hassan *et al.*, 2009 and 2011).

Exogenous enzymes are commonly used to improve the nutritive value of feeds for non-ruminants and as silage additives for ruminants. The use of crude enzyme to improve nutritive value of forage is not a new concept, first reported in the early 1960s, more recent developments in the enzyme industry have led to re-examination of the role of these enzymes and several comprehensive reviews have been published as McAllister *et al*.(1999); Beauchemin *et al*.(2003) and Krause *et al*.(2003).

Enzyme preparations containing high levels of cellulase, hemicellulase (xylanase) ,ligninase and pectinase have been used to improve the nutritive quality forages, nevertheless, most commercially available enzyme products are produced for non-feed applications and are mainly cellulases and xylanases (Bhat,2000). Cellulases and xylanases usually act synergistically to hydrolyze forage cell wall (Bhat and Hazlewood, 2001).

In this respect, Kung *et al*. (2000) argue that the addition of enzymes to feed may create a stable enzyme feed complex which protects the enzymes from ruminal proteolysis. Fibrolytic enzymes isolated from bacterial and fungal cultures increase the degradability during the process of ensilage of some forages. Besides, they improve *in vitro* digestibility of DM and NDF (Feng *et al*., 1996). So, a mixture of enzymes with various activities may be more effective (Yang *et al*., 1999). Moharrery *et al.* (2009) found when use two liquid exogenous enzyme with a rate of 1.0 g/kg of DM forage and allow for 24h at room temperature, improved ($p<0.05$) *in vitro* DM and NDF digestibility of white clover, red clover, alfalfa hay, perennial ryegrass compared with control and suggested that pre-treatment of forage with fibrolytic enzyme can solubility some fiber, so improve crude fiber digestibility. Exogenous fibrolytic enzymes might enhance attachment and/or improve access to the cell wall matrix by ruminal microorganisms and accelerate the rate of digestion (Nsereko *et al*.,2000). Adequate pre-incubation or pre-ingestion enzyme-substrate interaction seems necessary for improvement fermentation and digestibility of mature tropical grasses in ruminants by fibrolytic enzymes (Krueger and Adesogan, 2008) to allow, before feeding, a proper adsorption and binding of the enzyme to substrate, attachment and protection against degradation by rumen proteases (Beauchemin *et al.*, 2004a) and a stable enzyme feed complex (Fontes *et al*., 1995)

Feng *et al*. (1996) reported that pre-treatment of dry tropical grass with exogenous enzymes improved ($P< 0.05$) *in vitro* DM (43.5 vs. 38.7%) and NDF (31.1 vs. 26.0%) disappearance. Lewis *et al.* (1996) found that ruminal steers infusion enzymes caused lower disappearance of DM and NDF than enzyme application to the forage 24 h prior to feeding and suggested due to insufficient contact between enzymes and particulate substrate. Similarly, McAllister *et al.* (1999) found that ruminal infusion of a mixture of two commercial enzymes decreased DM and NDF digestibility versus addition to dietary silage in sheep.

Goosen (2005) reported appositive effects with Abo 374 (Abe 374 is a fungal enzyme cocktail containing cellulases, xylanases and mannanases with xylanase as major fibrolytic activity) enzyme on the *in vitro* DM and NDF degradation of wheat straw. Cruywagen and Goosen (2005) reported improved weight gain in lambs (6.75 and 7.13kg) and feed conversion ratios (0.15 and 0.16kg gain/kg DMI) when wheat straw was pre-treated with exogenous enzyme ABO 374 for 18h. before feeding at high and medium levels of enzyme application respectively. Hong *et al.* (2003) found nutrient degradation rate and effective degradability of DM,NDF and ADF increased with increasing enzyme level (0, 1 and 2%,w/w) and pre treatment times (0, 1, 12, 24h.) using goats and steers.

The aim of this study was to evaluate the effect different levels of exogenous fibrolytic enzyme treated to alfalfa hay (AH), barley straw (BS), common reed(R) and combination of these forages on its chemical composition and *in vitro* DMD and *in vitro* OMD using rumen liquor from sheep and goat.

MATERIAL AND METHODS

Forage samples

Alfalfa hay (*Medicago sativa*), Barley straw (*Hordeum vulgare*) obtained from Animal Resources Department farm at College of Agriculture, University of Baghdad. Common wild reed (*Phragmites communis*) was harvest from drainage river at the College of Agriculture, didn't exceed in height with two meter. Immediately after harvest reed, samples were oven dried (65 °C). Alfalfa hay (AH) was mixed with barley straw (BS) to formulate combination AH+BS, alfalfa hay (AH) was mixed with reed (R) to formulate combination AH+R and barley straw (BS) was mixed with reed (R) to formulate combination BS+R , all these combination was done with ratio 50:50(wt/wt) and alfalfa hay (AH) was mixed with barley straw (BS) and reed (R) to formulate combination AH+BS+R with ratio 33.33:33.33: 33.33(wt/wt/wt) thus we have 7 substrate of forages.

Enzyme products and cellulase assay:

The commercial multi-enzyme products in powder form used was Farmazyme (Farmvet, Turkey) containing per kg of enzyme preparation: cellulase (1,000,000 units), xylanase (1,500,000 units), ß-glucanase (100,000 units) and α-amylase (100,000 units) activities as indicated by the manufacturer.

Enzyme assays

Cellulase activity was measured as the concentration of reducing sugars by method of dinitrosalicylic acid (DNS) (Miller, 1959) as described by Mandels *et al*. (1976) and following the steps as described by Al-Ani (2005): DNS (3,5-dinitrosalicylic acid) reagent. Dissolve 1 g DNS in 30 ml of distilled water (DW) and add 20 ml of NaOH (2M). After complete dissolution, add 30g of Rochelle salts (sodium-potassium tartrate) gradually and slowly until total dissolution then complete to 100 ml with DW and keep DNS reagent in darkness at 4 °.C Glucose solution as in Whitaker and Bernhard (1972) of 10 *mM* (0.20 g of D-glucose in 100 ml of DW) to prepared standard glucose curve by used different tubes with different glucose concentration; 0, 0.2, 0.6, 1, 1.4, 1.8 and 2 mg/ml; 1 ml of DNS and boiled 5 min and tube cooled by tap water and 10 ml of DW was added to each tube and absorbance was measured at 540nm. The substrate was cellulose at 1% (1 g of cellulose in sodium acetate buffer 0.1 Molar, pH=5 and mixed well at least 2h. then complete volume to 100 ml with DW). The reaction mixture for each sample contained 0.9 ml of substrate and 0.1 ml of enzymic extract diluted (1:100).Test tube were used in duplicate and incubation was 60 min at 25, 30, 35 and 40 °.C in water bath, 1 ml of DNS was added and boiled 5 min, the tubes were cooled with tap water and absorbance was measured at 540 nm. For each sample blank with 0.9 ml of substrate was prepared incubated 60 min at same temperatures above , 1 ml of DNS and followed 0.1 ml of enzymic extract diluted (1:100) was added , boiled for 5 min , the tubes cooled with tap water and absorbance was measured at 540 nm. These steps were used to measured cellulase activity at different temperature, and the same steps were used above with temperature 35 °.C but with different buffer to measured cellulase activity in different pH by using 0.2 molar of phosphate buffer to achieve pH 5.5, 6.0, 6.5, 7.0 and 7.5 and each substrate was mixed well at lest 2 h and complete volume to 200 ml with DW. One enzyme activity (IU) was defined as the amount of enzyme required to release 1 μmol of reducing sugars as glucose per hour under experimental conditions described above.

The cellulase activity was 1.22, 0.89, 1.99, and 8.85 U/ml with specific activity of 0.22, 0.16, 0.36 and 1.59 U/mg for temperature 25, 30, 35 and 40 °.C and cellulase activity was 50.19,35.31,35.42,37.02,31.77 and 38.74 U/ml with specific activity of 9.03, 6.36, 6.38, 6.66, 5.72, and 6.97 U/mg.

Treatment of forage (Substrate of enzyme)

Air dry sample of alfalfa hay and barley straw and oven dry sample (65°C) of reed, milled to pass a 1.0 mm sieve. Based on the enzyme activity data, two enzyme activity showing high cellulase activity were selected. All treatment were carried out under 35°C and pH=5.5, without sterilizing the forages. The enzyme treated forages for 12 h with three levels of enzyme (1, 2, 3 g enzyme powder per 100g DM forage). At the end of treatment, samples were dried with oven (65°C) for 48 h, stored in small container for analysis.

In vitro digestibility procedures

Rumen liquor samples were obtained from two origin; Awassi sheep and black goats after slaughtered. Rumen liquor from each species separately, squeezed through four layer of cheesecloth (mesh size of 250µm) in to airtight container and transported to the laboratory. The strained rumen fluid kept in a water bath at 39 °C with CO_2 saturation, then mixed with buffer (artificial saliva) with a ratio10:40 ml prepared as described by Tilley and Terry (1963). During the first stage, a finely ground sample is incubated for 48 h with buffered rumen liquor in a glass tube under anaerobic conditions. In second stage the bacteria are killed by acidifying with HCl to pH 2.0 and are then digested by incubating them with pepsin for a further 48 h. Samples (0.5

g) of each forage (control or treated with one of the enzyme doses) were weighed in duplicate and placed in 200 ml test tubes, three tubes containing buffered rumen fluid and no forage sample were included within the incubation run and the mean value for these tubes was termed the blank value. The insoluble residue is filtered off, dried and ignited. The residue DM and OM is subtracted from the sample DM and OM to provide an estimate of DM and OM digested.

Chemical analysis

All proximate analysis was done according to AOAC (1995). Neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) were determined as described by Goering and Van Soest (1970) and hemicellulose was calculated as the difference between NDF and ADF. Cellulose was calculated as the difference between ADF and ADL.

Statistical analysis:

All data were analyzed using completely randomized design model (CRD). The chemical composition data were analyzed a (7 forages substrate× 4 level of enzyme) factorial design. *In vitro* digestibility data were analyzed following a (7 forages substrate×4 level of enzyme ×2 source of inoculums from sheep or goat rumen liquor) factorial design and means were separated using LSD, Duncan range tests using SAS (SAS , 2001).

RESULTS AND DISCUSSION

Biochemical activity

The results of the enzymatic profile indicated variation with other researcher (Colombatto *et al.*,2003a). The biochemical activities of feed enzymes tested under controlled, optimal condition does not always predict their ability to enhance ruminal feed digestion (McAllister *et al.,* 1999). Mainly, this is because the enzymes must work synergistically with ruminal microbial community to alert feed digestion (Morgavi *et al.,* 2000). Therefore, the selection of enzyme for use as ruminant feed additive should be first tested in a ruminal environment (Colombatto and Beauchemin, 2003) It also important to test enzyme on individual feeds, as their may be specific to the type of feed. The enzyme products were analyzed for their main and side activities at 39°C and pH 6.0, to reflect ruminal conditions (Colombatto *et al* 2003a) which apparently did not take place in our study,and the enzyme tested for cellulase activity only. Yang *et al* (2012) examined twenty six enzymes, these enzyme contaminated ranges of endoglycanase , xylanase , β-glycanase , α amylase and protease and found these enzyme contain arenge of crude protein and all enzymes had xylanase, endoglucanase and β-glucanase activity and their activity varied greatly.

Chemical composition

Chemical composition of alfalfa hay, barley straw, common reed and combination of these forages are presented in Table 1. The chemical composition of the base forages was different. Average values for the individual forages were within the range of values reported elsewhere (Hassan *et al.*2008; and Hassan and Tawfeeq, 2009), with small differences that could be attributed to varieties of forage, stage of maturity at harvest, weather conditions, soil type and management practices, as reported Moharrery *et al.* (2009). Nitrogen and ash contents in alfalfa hay were numerically greater than in barley straw and common reed. Barley straw and common reed had greater NDF and ADF content than alfalfa hay.

Enzyme treatment did not affect the content the CP content of any forage tested. This result disagrees with other results (Bata *et al.*, 2004; Yang et al, 2012). Bata *et al* (2004) use Cellpract AS100®, they found that enzyme treatment improves in crude protein content of rice bran and cotton seed meal. However, our results agree with Hong *et al.*,(2003); and Dean *et al.*, (2008). Dean *et al*. (2008) found ammonia treatment increase CP content of tropical grass but fibrolytic enzyme treatment not increase CP content. Although enzymes are protein, small amount of

enzyme typically added to aid fibrolysis of forage or diet is not sufficient to affect CP concentration (Hong *et al.*, 2003).

Enzyme treated decrease (P<0.01) NDF substrate content (Table 1), reducing ranging from 0.20-1.29% NDF, and decrease (P<0.01) ADF substrate content. These results would indicate that the pre- treatment with enzymes alerted the fiber structure, as previously reported in other studies (Nsereko *et al.*, 2000, Colombatto *et al.*,2003b; Giraldo *et al.*, (2007).

Several research (Nsereko *et al.*, 2000; Wallace *et al.*,2001; Giraldo *et al.*, 2007) have suggested that exogenous enzyme could increase fiber degradation through a hydrolytic action prior to feeding or *in vitro* incubation with rumen microorganisms.

Table (1): Effect of pre –treatment with different levels of enzyme on chemical composition of some forages.

Treatments		DM %	% on DM Basis							
Substrate	Enzyme	fresh	ASH	OM	CP	NDF	ADF	ADL	Hemi	Cell
Alfalfa hay (AH)	0	91.18^{d}	9.21^{d}	90.79^{a}	13.40^{a}	54.30^{a}	42.42^{a}	8.87^{a}	11.88^{a}	33.55^{a}
	1	94.49^{c}	12.22^{c}	87.78^{b}	13.39^{a}	53.59^{ab}	41.94^{a}	8.74^{a}	11.65^{a}	33.20^{a}
	2	96.44^{a}	15.59^{b}	84.41^{c}	13.41^{a}	53.01^{b}	41.43^{a}	8.56^{a}	12.23^{a}	32.21^{a}
	3	95.65b	22.07^{a}	77.93^{d}	13.41^{a}	53.19^{ab}	40.78^{a}	8.40^{a}	11.76^{a}	33.04^{a}
Barley Straw (BS)	0	93.54^{a}	11.08^{d}	88.92^{a}	2.89^{a}	81.88^{a}	52.10^{a}	11.59^{a}	29.78^{a}	40.51^{a}
	1	95.47^{a}	14.02^{c}	85.98^{b}	2.89^{a}	81.17^{a}	51.67^{a}	11.54^{a}	29.50^{a}	40.13^{a}
	2	96.32^{a}	21.61^{a}	78.39^{d}	2.90^{a}	81.50^{a}	51.95^{a}	11.52^{a}	29.55^{a}	40.43^{a}
	3	95.42^{a}	16.74^{b}	83.26^{c}	2.91^{a}	81.37^{a}	51.91^{a}	11.40^{a}	29.46^{a}	40.51^{a}
Reed (R)	0	93.49^{c}	8.97^{c}	91.03^{a}	4.22^{a}	77.85^{a}	52.61^{a}	35.33^{a}	25.24^{a}	17.28^{a}
	1	93.84^{b}	9.48^{c}	90.52^{a}	4.22^{a}	77.31^{a}	52.55^{a}	35.23^{a}	24.75^{a}	17.32^{a}
	2	94.61^{a}	11.97^{b}	88.03^{b}	4.23^{a}	77.06^{a}	52.51^{a}	35.22^{a}	24.54^{a}	17.30^{a}
	3	93.93^{b}	14.42^{a}	85.58^{c}	4.23^{a}	77.67^{a}	52.47^{a}	35.25^{a}	25.19^{a}	17.22a
Alfalfa hay + Barley straw (AH+BS)	0	92.30^{b}	10.33^{c}	89.67^{a}	7.79^{a}	67.21^{a}	46.94^{a}	9.92^{a}	20.26^{a}	37.02^{a}
	1	94.91^{a}	13.63^{b}	86.37^{b}	7.80^{a}	66.89^{a}	46.67^{a}	9.90^{a}	20.22^{a}	36.77^{a}
	2	94.66^{a}	20.30^{a}	79.70^{c}	7.79^{a}	66.94^{a}	46.47^{a}	9.86^{a}	20.41^{a}	36.62^{a}
	3	94.24^{a}	22.17^{a}	77.83^{c}	7.80^{a}	66.98^{a}	46.58^{a}	9.78^{a}	20.47^{a}	36.79^{a}
Alfalfa hay + Reed (AH+R)	0	91.17^{d}	9.38^{d}	90.62^{a}	8.79^{a}	64.83^{a}	47.36^{a}	22.24^{a}	17.46^{a}	25.12^{a}
	1	93.10^{c}	10.75^{c}	89.25^{b}	8.79^{a}	65.20^{a}	47.27^{a}	22.18^{a}	17.93^{a}	25.09^{a}
	2	93.60^{b}	14.14^{b}	85.86^{c}	8.80^{a}	64.18^{a}	47.16^{a}	22.08^{a}	17.02^{a}	25.08^{a}
	3	94.10^{a}	16.90^{a}	83.10^{d}	8.81^{a}	64.34^{a}	47.03^{a}	22.07^{a}	17.31^{a}	24.95^{a}
Barley straw + Reed (BS+R)	0	92.13^{c}	10.46^{c}	89.54^{a}	3.28^{a}	80.48^{a}	52.60^{a}	23.47^{a}	27.88^{a}	29.13^{a}
	1	93.56^{b}	12.74^{b}	87.26^{b}	3.29^{a}	80.16^{a}	52.48^{a}	22.67^{a}	27.68^{a}	29.81^{a}
	2	94.58^{a}	13.92^{b}	86.08^{b}	3.30^{a}	79.97^{a}	52.30^{a}	22.50^{a}	27.68^{a}	29.79^{a}
	3	93.50^{b}	16.72^{a}	83.28^{c}	3.30^{a}	80.20^{a}	52.37^{a}	22.79^{a}	27.84^{a}	29.57^{a}
Alfalfa hay + Barley straw +Reed (AH+BS+R)	0	91.57^{b}	9.83c	90.17^{a}	8.47^{a}	70.30^{a}	50.03^{a}	18.23^{a}	20.29^{a}	31.80^{a}
	1	93.83^{a}	13.43^{b}	86.57^{b}	8.48^{a}	70.07^{a}	49.86^{a}	18.14^{a}	20.21^{a}	31.72^{a}
	2	94.92^{a}	14.09^{b}	85.91^{b}	8.49^{a}	69.92^{a}	49.68^{a}	18.14^{a}	20.22^{a}	31.54^{a}
	3	93.71^{a}	22.46^{a}	77.54^{c}	8.50^{a}	69.90^{a}	49.68^{a}	18.05^{a}	20.23^{a}	31.64^{a}

*Means with different subscript in the same column differ (P<0.05). *P<0.05, **P<0.01.*

There is evidence that some enzymes applied to feed affect its composition, principally by increasing solubility of DM and NDF (Moharrey *et al* 2009), possibly releasing more nutrients that are then available to enhance microbial colonization of feed particles. Thus, a pre-incubation period is very important (Krueger and Adesogan, 2008) to allow, before feeding, a proper adsorption and binding of the enzyme to substrate, attachment and protection against degradation by rumen proteases and a stable enzyme feed complex (Beauchemin *et al.*, 2004a). The close association of enzyme with feed may enable some form of pre-ingestive attack of the

enzyme upon the plant fiber or enhance binding of the enzymes to the feed thereby increasing the resistant of the enzyme to proteolysis in the rumen (Beauchemin *et al.*, 2004b).Dean *et al* (2008) and Moharrey *et al* (2009) found enzyme treatment had different effect on cell wall component and forge type affected the response. The sterified bound between cellulose, hemicellulose and lignin restrict the digestion of cell wall by rumen microorganism; however, it has been shown (Nsereko *et al* 2000; Giraldo *et al* 2007) that exogenous fibrolytic enzyme could potentially improve fiber degradation through hydrolytic action prior to feeding or *in vitro* incubation.

In this study, the 12h pre-treatment with enzyme of seven forages substrate did not affect ($P>0.05$) their NDF content. These results would indicate that effectiveness of fibrolytic enzymes varies with the substrate (McAllister *et al.*, 1999). The ability of cellulases and xylanases to increase the extent of fiber digestion may be limited by the lack of enzymes that degrade the core structure of lignin-cellulose complexes in low quality forages. Cross- linking of lignin of the cell wall polysaccharides through ferulic acid bridges is key mechanism by which lignin limits cell wall digestion in plant by ruminants.

In vitro digestibility

Results of exogenous enzyme pre treatment of forages on in vitro DMD and OMD for sheep and goat rumen fluid are presented in Table (2).

In alfalfa hay, the pre-treatment with exogenous enzyme increase ($P<0.01$) *in vitro* DMD and OMD for sheep rumen fluid and *in vitro* OMD for goat rumen fluid. Perhaps the exogenous enzyme used in this study was work with more digestible structure such as good quality forage, may be its contain highest crude protein content than other forage tested. Increasing level of enzyme was significantly ($P<0.01$) for alfalfa hay only but not significant with other substrate.

In barley straw, increase in DMD and OMD in vitro was not significant, increasing level of enzyme treatment, slight, non significant increase *in vitro* DMD and increase *in vitro* OMD in rumen fluid from sheep and goat.

In reed, the pre- treatment with exogenous fibrolytic enzyme, has also slight increase *in vitro* DMD and increase *in vitro* OMD and non significant. Best values of *in vitro* DMD and OMD were with alfalfa hay (AH) , the lowest values were with wild reed (R) The differences in digestion between alfalfa hay ,barley straw and reed likely reflect differences in their composition , which reed containing greater amount of lignin .Additionally, the structure of reed my have impeded the full of enzyme. Same result has obtained with Yang *et al.* (2012) when used wheat straw and alfalfa hay with different enzyme.

Our results were disagree with other results (Jalilvand *et al.*, 2008). On the other hand, Colombatto and Beauchemin (2003) suggested that enzymes enhance alfalfa digestion by removing structural barriers retarding microbial colonization, thus increasing the rate of degradation.

It seems that the level of enzyme 3% had best improvement than other level (1 and 2%) in improving the *in vitro* DMD and *in vitro* OMD. Except improvement with alfalfa hay +barley straw (AH+BS) also there is non significant increase, but the improvement was higher with the level of enzyme 2% followed by improvement in Alfalfa hay +reed (AH+R), the same trend with level of enzyme 2%. Jalilvand *et al.*,(2008) indicated that the responses of level of Mutli-enzyme commercially available as feed additive named Naturzyme containing (per g of enzyme) preparation cellulase (4200 U), xylanase (2500U),β-glucanase(500U), protease(3000 U) and amylase (750 U) activities enzyme (0, 3, 6,and 9 g of enzyme /Kg of DM forage) addition differ with forage type(alfalfa hay , maize silage , wheat straw), so that the addition was more effective with more fibrous roughage such as wheat straw, an important factor determining the efficacy of enzymatic treatment of forages is the level of enzyme application and it was observed that high levels of addition can be less effective than low levels. These effects were favorable enhancing straw digestion with a low level of enzyme addition (3 g enzyme/kg DM), whereas high levels of enzyme addition seemed to affect adversely

fermentation rate of straw. (Jalilvand *et al.*, 2008). Moreover, Nsereko *et al.* (2000) reported that relatively low levels of enzyme supplementation increased numbers of both fibrolytic and non-fibrolytic bacteria in the rumen and concluded this effect may be due to release of polysaccharides that are utilized by these bacteria. However, the increase in bacterial Numbers was not apparent when high levels of enzyme. This variation in nutrient digestibility results observed by fibrolytic enzyme may be due to many factors such as forage level, enzyme level, method of enzyme application (Colombatto *et al* .,2003, Beauchemin *et al.*,2003).The pre-incubation period is very important to allow, before feeding, a proper adsorption and binding of the enzyme to substrate, attachment and protection against degradation by rumen proteases (Beauchemin *et al.*,2003) According to Beauchemin *et al.* (2004), enzymes are most effective when added to feed in liquid for prior to feeding. Therefore, adequate pre-incubation or pre-ingestion enzyme - substrate interaction seems necessary for improvement of fermentation and digestibility of mature tropical grasses in the ruminants by fibrolytic enzyme.

Table (2): Effect of forages pre -treatment with different levels of enzyme on *in vitro* DMD and OMD using sheep and goat rumen fluid.

		Sheep		Goats	
Forages(Substrate)	Level of Enzyme	DMD	OMD	DMD	OMD
Alfalfa hay (AH)	0%	53.43^{c}	54.18^{b}	52.60^{a}	53.44^{b}
	1%	54.11^{bc}	54.78^{ab}	53.26^{a}	54.02^{ab}
	2%	55.77^{ab}	56.15^{ab}	54.89^{a}	55.91^{a}
	3%	56.19^{a}	56.58^{a}	55.15^{a}	55.89^{a}
Barley Straw (BS)	0%	43.23^{a}	44.47^{a}	43.22^{a}	44.75^{a}
	1%	43.38^{a}	45.25^{a}	43.29^{a}	45.25^{a}
	2%	44.21^{a}	45.64^{a}	44.21^{a}	45.71^{a}
	3%	44.48 ^{a}	45.94^{a}	44.43^{a}	45.96^{a}
Reed (R)	0%	30.70^{a}	30.95^{a}	31.00^{a}	31.61^{a}
	1%	30.85^{a}	31.30^{a}	31.19^{a}	31.96^{a}
	2%	31.47^{a}	31.87^{a}	31.80^{a}	32.48^{a}
	3%	31.53^{a}	31.80^{a}	31.98^{a}	32.70^{a}
Alfalfa hay +barley straw (AH+BS)	0%	47.33^{a}	47.86^{a}	47.63^{a}	48.93^{a}
	1%	47.75^{a}	48.20^{a}	48.11^{a}	49.26^{a}
	2%	48.65^{a}	49.09^{a}	49.00^{a}	50.70^{a}
	3%	49.04^{a}	49.05^{a}	49.35^{a}	50.80^{a}
Alfalfa hay +reed (AH+R)	0%	40.68^{a}	40.96^{a}	40.74^{a}	41.22^{a}
	1%	41.11^{a}	41.33^{a}	41.18^{a}	41.67^{a}
	2%	41.65^{a}	41.90^{a}	41.75^{a}	42.25^{a}
	3%	41.91^{a}	42.10^{a}	42.05^{a}	42.29^{a}
Barley straw +reed (BS+R)	0%	36.94^{a}	37.28^{a}	37.44^{a}	38.16^{a}
	1%	37.06^{a}	37.43^{a}	37.56^{a}	38.23^{a}
	2%	37.65^{a}	37.99^{a}	38.19^{a}	38.50^{aa}
	3%	38.02^{a}	38.17^{a}	38.51^{a}	39.41^{a}
Alfalfa hay + barley straw + reed (AH+BS+R)	0%	42.19^{a}	43.07^{a}	42.21^{a}	42.23^{a}
	1%	42.23^{a}	43.17^{a}	42.27^{aa}	42.93^{a}
	2%	42.85^{a}	43.71^{a}	42.90^{a}	43.00^{a}
	3%	43.26^{a}	44.04^{a}	43.28^{a}	42.91^{a}
Level of enzyme	0%	42.07^{a}	42.68^{a}	42.12^{b}	42.91^{b}
	1%	42.36^{a}	43.06^{a}	42.41^{ab}	43.33^{ab}
	2%	43.18^{a}	43.76^{a}	43.25^{ab}	44.08^{ab}
	3%	43.49^{a}	44.01^{a}	43.54^{a}	44.31^{a}

*Means with different subscript in the same column differ (P<0.05), .*P<0.05, **P<0.01.*

Nevertheless, feed enzymes have been used to improve the use of a wide range of diets containing legumes, grasses, haylage, straw and other feedstuffs (Beauchemin *et al.*,2003). The

mode of action of these enzymes in ruminants is not fully understood. They can enhance feed colonization by increasing the numbers of ruminal fibrolytic microbes (Morgavi *et al.*,2000; Nsereko *et al.*,2000) and thus can increase the rate of degradation in the rumen (Yang *et al.*,1999). Another important reason for applying enzymes to feed prior ingestion to enhance binding of the enzyme to the feed, thereby increasing the resistance of the enzyme to proteolysis in the rumen .Enzyme applied to feed prior to ingestion are particularly stable ; the presence of substrate is known increase enzyme resistance to proteolytic inactivation (Fontes *et al.* 1995)

In the present study, the lack of effect of enzyme on forage NDF and ADF content was in agreement with the observed inefficiency of enzymatic treatment to increase forage *in vitro* DMD and OMD

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تاثير الهضم الاولي للاعلاف الخشنة المتوفرة محلياً بالانزيمات المحللة للالياف في التركيب الكيميائي ومعامل الهضم المختبري

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اجريت هذه الدراسة لمعرفة تاثير الهضم المسبق بالانزيمات المحللة للالياف لبعض الاعلاف الخشنة المتوفرة محلياً مثل دريس الجت، تبن الشعير، القصب البري ومزيج منه وذلك لزيادة كفاءة الاستفادة منها. تمت معاملة هذه الاعلاف لمدة 12 ساعة بالمستويات 0، 1، 2 و 3% من خليط الانزيم التجاري Farmazyme الذي يحتوي على مزيج من الانزيمات.

اظهرت نتائج التحليل الكيميائي للاعلاف الخشنة المعاملة بالانزيم زيادة عالية المعنوية ($P<0.01$) في محتوى المادة الجافة والمادة غير العضوية (الرماد)، مع انخفاض عالي المعنوي ($P<0.01$) في محتوى المادة العضوية ومستخلص الالياف المتعادل وعدم تاثر اللكنين ومحتوى البروتين الخام في تلك الاعلاف بالمعاملة.

اظهرت نتائج تجربة الهضم المختبري زيادة عالية المعنوية ($P<0.01$) في معامل الهضم المختبري للمادة الجافة والمادة العضوية لهضم دريس الجت فقط مع سائل كرش من الاغنام وان استخدام سائل كرش الماعز ادى الى زيادة عالية المعنوية في معامل هضم المادة العضوية فقط لدريس الجت.

الاستنتاج: تفوق معامل هضم المادة الجافة والعضوية لدريس الجت المعامل بالانزيم مقارنة مع غير المعامل، بينما لم يتأثر القصب البري بالمعاملة المسبقة بالانزيم. لم يكن هناك تاثير معنوي لمستوى الانزيم المستخدم بالمعاملة على معامل هضم المادة الجافة او المادة العضوية مع تفوق حسابي للمستوى 3% كما لم يوجد أختلاف معنوي في معامل هضم المادة الجافة او العضوية للاعلاف الخشنة عند استخدام سائل كرش الاغنام او الماعز في تجربة الهضم المختبري.

الكلمات المفتاحية *: دريس الجت، تبن الشعير، القصب البري، معامل الهضم المختبري.*