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Determining of Degradation Parameters of Sorghum Silage with Different Levels of Fibrolytic Enzymes Using in Situ Technique

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ABSTRACT

This experiment was conducted to determine of nutritive value, chemical composition and digestibility sorghum bicolor silage with different levels of fibrolytic enzymes using in situ technique. Chemical compositions were measured according to the standard methods. Three fistulated-Baluchi male lambs used factorial experiment in a completely randomized design. Treatments were A: sorghum silage, B: sorghum + 3g fibrolytic enzymes before silage, C: sorghum + 6g fibrolytic enzymes before silage, D: sorghum + 9g fibrolytic enzymes before silage, E: sorghum + 3g fibrolytic enzymes after silage, F: sorghum + 6g fibrolytic enzymes after silage and G: sorghum + 9g fibrolytic enzymes after silage. The amounts of degradation were measured using nylon bags at 3, 6, 9, 12, 24, 36, 48, 72 and 96 h times. The degradability parameters of dry matter for potential degradability fractions (a+b) were 60.77, 61.52, 64.43, 68.56, 62.60, 63.04 and 66.95% for treatments, respectively. The results showed significantly differences in degradability of experimental treatments in different incubation times and treatment D had highest and treatment A had lowest rumen degradability of 96 h. The result showed that enzymes were causes the significant reduction of NDF and ADF and significant increase of CP, EE and WSC in silage. Between the enzymes is added before or after the silage process can be concluded that in both cases, the enzyme causes significant changes in the treatments containing silage compared to the control treatment. So, this process for feeds in the local animals are benefit and nutrition value of them is suitable, therefore we can recommend them for providing part of roughage requirements in animal feed.

Keywords: Degradability, Enzyme, In situ, Nutritive Value, Sorghum.

INTRODUCTION

Supply of protein is required for every country through livestock products is the basic policy of agriculture, that by cultivation of high yielding forage plants is possible and reduces

the pressure on rangeland and pasture (Koochaki, 2007). Sorghum in form of hay or silage is used in animal feed. Varieties of sorghum are useful to use in poultry, breeding pig and other animal feeds and the nutritional value of sorghum is known in much of the research equal with corn (Salari, 2012).

The main objective of maintaining agricultural products is the keeping of optimum conditions for use in seasons that not exist, which one of these methods is silage. Although, to date, preparation of hay forages has improved by use of new drying techniques, but since these methods are highly specialized and much time is spent, so many ranchers prefer to maintained of forages to the silage. Also, many anti-nutrient substances in plant are losses by silage (Valizadeh *et al*., 2007).

Enzymes reduce the activation energy of the reactions that cause of reaction progress. Enzymes are extremely sensitive to the environment conditions and can be present best performance in the suitable pH and temperature (Sadeghi and Shawrang, 2007). The main reason of the use of enzymes is to improve the nutritional value of feed. The efficiency of natural digestion process is not complete, so adding enzymes to feed caused the increase of efficiency and digestion (Salari, 2012). Many researchers believe the negative relationship between digestibility and concentration of phenolic chemical compounds,which present in the forage cell walls (Ghorbani, 1995). Digestibility of dry matter of sorghum had the negative correlation with tannin content and varieties of sorghum due to the differences in tannin content have the different dry matter digestibility (Ebadi, 1996). Silage process has the great effect on quality of forages (Valizadeh *et al*., 2003). Silage reduces the amount of tannin and increased of dry matter digestibility of sorghum (Salari, 2012).

MATERIAL AND METHODS

Sorghum Collection

Sorghum bicolor was obtained from local variety of department of agriculture of *Kashmar, Iran*. Sampling was carried out the aerial parts of the plant (10 cm above the ground). Samples were taken out of the containers and dried in the sunlight and milled in 2 mm size to be used in other phases of the experiment and were silage in plastic buckets, under laboratory temperature. Animals used in this experiment were fed at maintenance level. The animals were fed with a mixture of 60% forage and 40% concentrate diet (Ørskov and McDonald, 1979). Experimental treatments were A: sorghum silage, B: sorghum + 3g fibrolytic enzymes before silage, C: sorghum + 6g fibrolytic enzymes before silage, D: sorghum + 9 g fibrolytic enzymes before silage, E: sorghum + 3g fibrolytic enzymes after silage, F: sorghum + 6g fibrolytic enzymes after silage and G: sorghum + 9g fibrolytic enzymes after silage. After 45 days, the silages were opened and silages pH was measured with pH meter (Polan *et al*., 1998). Then, the samples were milled and were used for experiments to determine the approximate analysis of chemical compounds.

Chemical Composition

Feedstuff dry matter (DM, method ID 934.01), ash (method ID 942.05), ether extract (EE, method ID 920.30), and crude protein (CP, method ID 984.13) were determined by procedures of AOAC (1999). The neutral detergent insoluble fiber (NDF) and acid detergent insoluble fiber (ADF) concentrations were determined using the methods of Van Soest *et al*., (1991), without sodium sulphite. Neutral detergent insoluble fiber was analyzed without amylase with ash included.

Measured In Situ

To estimate the degradability of the nylon bag technique, the feed samples were milled with a special mill and 2 mm sieve (Moghaddam *et al*., 2012). 5 gram of each nutrient was poured into bags made of synthetic polyester fiber as 6 × 12 cm and pore diameter of 50 mm.

Three fistulated-Baluchi male lambs used factorial experiment in a completely randomized design. Incubation times were 3, 6, 9, 12, 24, 36, 48, 72 and 96 h. After each incubation time, the bags were removed and rinsed with cold water until the water is completely cleared out. After washing, bags were incubated for 24 h at a temperature of 65 °C to evaporate and for 24 h at 105 °C in oven (Moghaddam *et al*., 2012). Degradation parameters (soluble, insoluble and fixed rate of degradation) were calculated with Naway and Fitcurve. For matched degradation data used from $P=a+b(1-e^{-ct})$ that a=The degradation of soluble fraction (%), b=The degradation rate of insoluble fraction (%), c=The constant degradation rate (%/h), t=The incubation time (h), e=The constant factor (2.718) and P=The degradation rate at the time (t). Effective degradability was calculated at ED=a+(b×c)/(c+k) that (k) is passage rate which were considered in this study 0.02 (Ahmadi *et al*., 2013).

Statistical Analysis

The obtained data from in situ study was analyzed according to factorial experiment in a completely randomized design with 4 replicates by the GLM procedure (SAS, 1996). The treatment means were compared by the Duncan test.

RESULTS AND DISCUSSION

Chemical compositions of treatments are presented in Table (1). The data show that treatment G had the most (21.56%) and treatment F had the least (19.35%) amount of dry matter.

Table 1: The chemical composition of feeds (% DM)*

Treatments	DM	CP	NDF	ADF	Ash	OM	EE	WSC	pH
A	19.85^{b}	8.33^{c}	68.30^{a}	41.90^{a}	18.27^{c}	81.72^{a}	2.17^{c}	2.21^{b}	4.10^{abc}
B	21.41^{a}	9.11^{a}	65.56^{b}	41.12^{b}	19.23b^{c}	80.76^{ab}	3.41^{a}	2.42^{a}	4.20^{a}
C	19.71^{b}	7.30^{d}	64.94b^{c}	39.45^{d}	19.08b^{c}	80.91^{ab}	2.46^{c}	2.46^{a}	3.90^{bc}
D	20.14^{b}	8.29^{c}	63.74^{de}	38.30^{e}	19.41b^{c}	80.58^{ab}	2.88^{b}	2.49^{a}	4.10^{abc}
E	19.78^{b}	8.32^{c}	64.77^{c}	40.24^{c}	19.24b^{c}	80.75^{ab}	2.25^{c}	2.42^{a}	3.83^{c}
F	19.35^{b}	8.74^{b}	63.98^{d}	39.28^{d}	19.99^{ab}	80^{bc}	1.28^{d}	2.50^{a}	4.20^{a}
G	21.56^{a}	8.80^{b}	63.19^{e}	38.67^{e}	21.48^{a}	78.51^{c}	2.19^{c}	2.48^{a}	4.16^{ab}
SEM	0.201	0.066	0.130	0.077	0.732	0.732	0.051	0.012	0.020
A	**	**	**	**	*	*	**	**	*NS*
S	*NS*	**	**	*NS*	**	**	**	*NS*	*NS*
*A*S*	*NS*	**	*NS*	**	*NS*	*NS*	**	*NS*	*NS*

*DM=Dry matter, CP=Crude protein, NDF=Neutral detergent fiber, ADF=Acid detergent fiber, OM=Organic matter, EE=Ether extract, WSC=Water soluble carbohydrate, pH=Power hydrogen, SEM=Standard error means of the difference amount three treatments means, A=Effect of enzyme, S=Effect of silage, A*S=Effect of enzyme and silage.

a,b,c,d,eWithin a column, means without a common superscript letter differ (P< 0.05).

It can be seen, among of treatments in the dry matter has not been regular changes and enzyme is not caused to reduce or increase the dry matter of treatments regularly. Some researchers reported the dry matter of maize silage was not change affected by additive enzyme (Higginbotham *et al*., 1994; Stokes and Chen, 1994), and some scientists have concluded the dry matter reduced (Chen *et al*., 1994; Sheperd and Kung, 1996), which this decrease may be due to the product of acetic acid or ethanol in the silage (Spoelstra *et al*., 1992). Some scholars have stated, add enzyme have no effect on the silage dry matter (Colombatto *et al*., 2003), that these results are similar to results obtained in the present study.

Amount of crude protein of treatments (Table 1), show the lowest crude protein was in treatment D (8.29%) and the highest level was in treatment B (9.11%), which show add enzyme causes of increase crude protein of silage. The crude protein changes caused by increased levels of the enzyme shows that increased levels of the enzyme no systematic

changes in silage crude protein whether before or after opening the silage. The researchers stating the increased levels of enzymes in straw silage were not significant effect on the crude protein (Shahsavani, 2009). Some of other researchers showed added the enzymes caused to increased crude protein of sorghum straw silage (Xing *et al.,* 2009). Also, researchers reported cellulose enzymes from *Trichoderma* fungi were inactivated in the rumen with activity of rumen bacterial protease enzyme (Kopecny *et al*., 1987), so reported unprotected additive enzymes is rapidly disabled in the rumen (Chesson, 1994), but some studies show (Hristov *et al*., 1998; Morgavi *et al*., 2000), several additive enzymes are active in the rumen, especially these added to diet before feeding (Fontes *et al*., 1995). The changes of treatments pH present in Table (1). As it is clear, added enzyme before or after silage do not induces pH changes in the silage. So, the lowest and highest pH values were for the treatment E (3.983%) and the treatments B and F (4.2%) respectively, and changes in pH show no relation regular with increased enzyme levels. In one study, the effect of adding mixed enzyme on the degradation of polysaccharides on chemical commotions and digestion of rye grassand alfalfa silage were examined and the result show the additive enzyme increased lactic acid which this cause of decreased pH in the silage (Zha *et al*., 1999).

Some scholars were investigated the effect of adding the lactic acid bacteria or α-zaym enzyme (containing cellulase, amylase and pectinase enzymes) to first harvest of alfalfa before silage and concluded the adding enzymes to improve the fermentation and reduced pH in the silage (Shepherd and kung, 1996). Most investigators have reported adding fiber-degrading enzymes were not effect on final pH in the silage (Higginbotham *et al.,* 1994*;* Stokesandchen, 1994). The reason of this lack of pH changes in the silage has sufficient amounts of fermentable carbohydrate and the effect of enzymes does not convert the extra carbohydrate to the organic acids (Colombatto, 2000).

The influence of defferent levels of additive enzymes on the silage pH changes not been investigated thoroughly, but some researchers believe that increased levels of enzymes has little effect in the pH change on the silage (Spoelstra *et al.,* 1992; Sheperd and Kung, 1996). But some reports of mixed fermentation of *Trichoderma Viride* fungi caused to change final pH of corn silage. As respects the forage sorghum were harvested in mid-flowering, so concluded no pH change in the silage can be due to sorghum harvesting stage (Autrey *et al.,* 1975).

Basically, the most effect of enzyme was in the cell wall and cell wall without hemicellulose. In Table (1), present the results of changes in the cell wall by enzyme effect. As is observed, the maximum content of cell wall was the control (68.30%) and the least amount was for the treatment G (63.19%). Treatments A (41.9%) and D (38.30%) had the highest and lowest amount of the cell wall without hemicellulose. According to results of the present experiment, increase the enzyme levels in treatments which the enzyme is added before or after opening the silage, caused decreased cell wall and cell wall without hemicellulose of treatments compared to the control. In a separate study showed the decrease of ADF and NDF by use of fiber degradation (Koc and Coskuntuna, 2003).

Some researchers, concluded the degradation act of enzyme with increasing enzyme level when increases that increasing the amount of available substrate (Nidetzky *et al*., 1993). Various studies suggested with increasing enzyme levels, decrease the amount of available substrate and action site of the enzyme is saturated and therefore, enzyme degradation does not increase linearly. Many researchers studied the effect of enzymes degradation on forage legume (Iwaasa *et al*., 1997; Jaster and Moore, 1998; Nadea and Buxten, 1997), and mixture of grasses and legume forages (Henderson and McDonald, 1977). Most reviews show decreases fiber of forage silage by adding cellulase and hemi-cellulase or mix of them enzymes.

As in the Table (1), observed among treatments which enzymes received, increase in the levels of enzyme is causing significant difference in the cell wall ($P<0.05$). So that, average and high levels of enzyme show most of the cell wall decrease. The decrease in cell wall by

increasing the levels of enzymes indicates that although increased levels of the enzyme further reduces the cell wall, but the decline most felt at lower levels. According to Table (1), intermediate levels compared to the low levels of the enzyme shows a lot of changes to the cell wall, but due to the high level of cell wall enzymes causes more decrease than the average levels, however this reduction is not very sensible. In other words, the above results show the increased enzyme levels not affect linearly on the amount of enzyme. This result in the cell wall without hemi-celluloses was similar to the cell wall. Results of various reviews show that one of the most important factors in the use of enzymes is the amount of enzyme. Many different results in the use of enzymes in ruminant nutrition is obtained because of used insufficient or more than enough amounts of enzymes (Beauchemin *et al*., 2003).

The lack of effect of small amount of enzymes in some circumstances it may be due to the lack of appropriate enzyme activity. But cause of in effectiveness of the high levels of the enzymes are not clear (Beauchemin *et al*., 2000). Some scientists believe the average amount of additive enzyme levels creating appropriate changes in the cell wall degradation, but large quantities of enzyme levels by binding to feed particles, reduces the amount of microbial binding, so the rate of digestion is affected. Obviously, with increasing the enzyme levels amount of available substrate is decrease (Nsereko *et al*., 2000) and degradation act of enzymes does not increase linearly.

As shown in Table (1), the different enzyme levels have difference significantly affect in the time of silage for the treatments, and in the treatments that received enzyme after opening the silage, further reduced the cell wall, which this could be due to the tannins in the silage that has inhibitory effects on enzyme act. Some hay and forages silage contain the materials which reduce the effect of enzymes. Tannins and polyphenols compound known as anti-nutritional factors which can reduce the effect of degrading polysaccharides enzymes (Barahona *et al*., 2006). This inhibitor effect about xylanase enzyme had been reported by Nsereko *et al*., (2000).

Excessive moisture of feed caused to occurs non-uniformity in the influence of enzymes. Therefor the use of enzymes in dry feed can remove the non-uniformity effect of enzyme (Beauchemin *et al*., 2003). Some experiments showed the enzymes had more effective in dry forage compared to wet forage (Beauchemin *et al*., 1998). Evidence show the enzymes effective in the chemical composition of feed before consumption by animal, due to the release of soluble sugars (Hristov *et al*., 1996) and in some feed caused to soluble the part of NDF and ADF (Christensen, 1997, Krause *et al.,* 1998) and have reported adding enzymes to feed, created structural changes in plant which increased the degradability of them (Salari, 2012).

Adding enzymes to ration increased the power of the rumen hydrolysis by interactive effects with the rumen bacteria that causes increased enzyme effect in the rumen and increased the total digestibility which this results show the fiber degradation enzymes can be effective when added to the feed (Yang *et al*., 1999; Morgavi *et al*., 2000; Wang *et al*., 2001).

Dry Matter Degradation at Different Incubation Time

The results of the dry matter degradation of treatments are presented in Table 2. As can be seen, in all treatments were significant differences observed among of control and treatments containing enzyme, it can be deduced the enzyme in all treatment is caused to increase dry matter degradation significantly ($P<0.05$). In the all times of incubation, the medium and high levels of enzyme show highest dry matter degradation. According to the results specified, with most of the incubation time, increases the dry matter degradation.

From the data of Table (2), it can be concluded that low amount of NDF and ADF in the treatments containing enzyme is caused to increase dry matter degradability compared to the control treatment (Feng *et al*., 1996). Positive effects of additive enzymes in experiment by nylon bags method has confirmed and obtained the increased nutrient digestibility of forages and increase the disappearance of dry matter and NDF from dry forages (Lewis *et al*., 1996).

Researchers have added the mixture enzymes to the dry grass in the nutrition of fattened calves and concluded the digestibility of DM, NDF and ADF in the entire of digestive system is increased to extent the 5.5, 8.9 and 13% (Feng *et al*., 1996).

Table 2: Means of dry matter degradation of feeds by incubation at different times in the in situ method (% DM)

Treatment	Incubation times (h)						
	3	6	12	24	48	72	96
A	24.89^{f}	27.99^{f}	31.15^{d}	45.29^{d}	50.63^{e}	55.58^{e}	60.00^{e}
B	26.95^{e}	32.15^{d}	37.89^{c}	48.47^{c}	55.04^{d}	59.43^{d}	61.64^{d}
C	33.53^{c}	37.84^{b}	41.14^{b}	51.92^{b}	58.64^{bc}	61.58^{bc}	64.09^{b}
D	36.35^{a}	39.90^{a}	42.12^{b}	55.95^{a}	61.09^{a}	65.32^{a}	67.96^{a}
E	27.26^{e}	30.79^{e}	37.08^{c}	48.05^{c}	54.48^{d}	6,.40^{cd}	61.63^{d}
F	31.47^{d}	35.21^{c}	42.46^{ab}	52.20^{b}	57.81^{c}	62.20^{b}	62.97^{c}
G	34.77^{b}	39.72^{a}	43.99^{a}	55.54^{a}	59.70^{b}	65.34^{a}	67.06^{a}
SEM	o.493	0.243	1.13	0.841	0.871	0.820	0.551
A	**	**	**	**	**	**	**
S	**	**	*NS*	*NS*	*	*NS*	*NS*
*A*S*	**	**	*NS*	*NS*	*NS*	*NS*	*NS*

SEM=Standard error means of the difference amount three treatments means, A=Effect of enzyme, S=Effect of silage, A*S=Effect of enzyme and silage.

a,b,c,d,e Within a column, means without a common superscript letter differ (P< 0.05).

Degradation capacity in the rumen is a lot, so effect of enzymes in rumen can be due to the effect of reinforcing additive enzymes to the microorganisms enzymes in the rumen (Morgavi *et al*., 2001), which this positive effects can be result of cooperation between these sections. The final result of this collaboration is increased the amount of enzyme activity that is greater than the effect of each section. However, methods which determined the how to enzymes effect are not accurately, despite the high digestibility of starch and fiber in rumen. Recent studies have been specified, part of the enzyme act (Beauchemin *et al*., 2004).

Dry Matter Degradability Parameters

The results obtained from analysis of degradation parameters of studied plants show in Table (2). As can be observed, the most degradability in (a) fraction (component of fast degrade) was the treatment D (32.17%) and lowest was control (20.18%). In terms of (a) fraction observed significant difference among control and treatments contain enzyme (P<0.05), and the enzyme in all treatments causes to increase of (a) fraction. The high and middle levels of the enzyme were best levels. The control treatment (34.51%) was lowest and the treatment E (39.96%) was highest amount of (b) fraction (slow degrade fraction). In all treatments, observed significant differences among control and treatments contain enzyme (P<0.05), and increased enzymes levels lead to increase of (b) fraction. Salari (2012), showed the cellulase enzyme, in the first stage attacked to fast digestion part of plant and residual part will be less digestibility.

Increased levels of enzyme was caused increase (a+b) fraction (effective degradability). Significant differences were observed among treatments with the identical levels of enzyme but differed at stages of added to silage (P<0.05). In the (c) fraction were observed significant difference among control and treatments contain enzyme (P<0.05), and enzyme was caused to increase of (c) fraction (constant rate of degradability).

Deficiency in (a) fraction is caused to slow growth in the rumen microorganisms, which cause of reduce feed degradability in the rumen (Salari, 2012). Due to, (a) fraction is smaller and (b) and (c) fractions are more, so better, to sorghum silage containing low levels of the enzyme in before and after opening silage in order to see the best performance. Whatever the constant rate of (c) fraction is higher, expected to feed intake increases (Danesh Mesgaran *et al.*, 2008). The mean of effective degradability of dry matter per hour (0.02, 0.05 and 0.08)

were significant different among of all treatments containing enzyme and control (P<0.05), and increase enzyme levels in all treatments caused to increase the dry matter degradability. Also, increased enzyme levels were significantly caused to increase dry matter degradability (P<0.05), so the treatments containing high levels of the enzyme, showed the highest dry matter degradation.

Table 3. The parameters estimated from the dry matter degradability coefficients of feeds

Treatments	Degradation coefficients				ED=0.02	ED=0.05	ED=0.08
	A	B	C	a+b			
A	20.81^{e}	34.51^{c}	0.032^{d}	60.77^{e}	45.37^{d}	36.37^{f}	32.20^{f}
B	22.85^{d}	39.69^{a}	0.042^{b}	61.52^{e}	49.07^{c}	40.52^{d}	36.20^{d}
C	29.92^{b}	38.67^{a}	0.038^{bc}	64.43^{c}	52.57^{b}	44.92^{b}	40.85^{b}
D	32.17^{a}	36.36^{b}	0.036^{c}	68.56^{a}	55.57^{a}	47.42^{a}	43.37^{a}
E	22.90^{d}	39.96^{a}	0.038^{bc}	62.60^{d}	45.65^{d}	40.05^{e}	35.67^{e}
F	26.52^{c}	36.51^{b}	0.047^{a}	63.04^{d}	52.20^{b}	44.30^{c}	40.15^{c}
G	31.09^{ab}	35.85^{b}	0.041^{bc}	66.95^{b}	55.20^{a}	47.27^{a}	43.25^{a}
SEM**	0.650	0.763	0.0011	0.458	0.096	0.098	0.107
A	**	**	*	**	**	**	**
S	**	*	*NS*	*NS*	**	**	**
*A*S*	**	*	**	**	**	*NS*	*NS*

a=Dry matter solution at zero time (%), b=Fermentable material (%), c=Constant degradability coefficients at time t (%/h), a+b=Potential degradability, ED=Effective degradation (The passage of time, r=0.02), SEM=Standard error means of the difference amount three treatments means, A=Effect of enzyme, S=Effect of silage, A*S=Effect of enzyme and silage.

a,b,c,d,e Within a column, means without a common superscript letter differ (P< 0.05).

In general, can be concluded from degradability of dry matter and its parameters, that high fiber and low protein content in control treatment compared to treatments contain different enzyme levels, leads to slow degradation of dry matter and this parameters of it (Takasy *et al*., 2007).

CONCLUSION

The purpose of this study was to survey the different effects of fibrolytic enzymes on nutritional value of sorghum bicolor silage, before and after opening the silage. To date, sorghum widely used for animal consumption, because this plant has a condition such as drought resistance and high production per unit. Silage this forage causes to increased forage durability and prevent shortage of fodder in the cold seasons for ranchers. Due to high fiber in the sorghum silage, its intake reduces in livestock and seems imperative to find ways to reduce sorghum fiber. Also, various additive enzymes are used in order to improve the quality and performance of silage fermentation. One of these additives is fiber-degradation enzymes (cellulase and xylanase). These enzymes added to reduce fiber, also caused to improve the non-structural carbohydrates, proteins, fats and fermentation in the silage. It is important to find the appropriate levels of the enzyme, because of different results are obtained in the use of enzymes in the ruminant nutrition, as a result of used insufficient or excessive amounts of enzymes (Salari, 2012). Knowing the enzyme is added the silage before or after opening it, can be useful. The results of this study showed the enzymes were caused decreased significantly of NDF and ADF, and significant increase in CP, EE and WSC in the silage and the best performance about enzyme levels were medium and high levels of enzyme. Between the enzyme added before or after the opening of silage, could be concluded in both cases the enzyme leads to significant changes in the silage compared to the control treatment. But in some cases, increase of enzyme after silage showed better performance, because enhance enzyme before silage due to high humidity is caused lack of uniformity of enzyme effects in the different parts of the silage.

In the present study, the enzyme is caused to increase the dry matter degradability, but added enzyme before and after silage not show significant changes. Level of medium and high of enzyme, was better than low level and showed significant differences. But increased enzyme level did not rise linearly, with the extent of this effect, that cause of this result is not clear exactly, but such factors as resistance remaining substrate versus the action of the enzyme effect by increasing the concentration of enzyme, prevent connection rumen microorganisms to fiber particles, are effective. Also, the large amount of enzyme causes the release of anti-nutritional factors (phenolic compounds), and high levels of phenolic compounds reduces microbial digestion (Moghaddam *et al*., 2012).

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