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Full Length Research Paper

Effect of fungal treatment on chemical composition and *in vitro* ruminal digestibility of some agricultural residues

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This study was conducted to investigate the effects of *Trichoderma harzianum* isolate T.447 (T.447) on the chemical composition and *in vitro* ruminal digestibility of treated maize, wheat, rapeseed and soybean straws. Preparation of each straw was divided into two equal parts and was treated with a suspension of T.447 spore or an equal volume of distilled water (control). Treated straws were placed in closed plastic bags and were incubated in a growth chamber at 25±1°C for 45 days. *In vitro* ruminal digestibility was determined after incubating the samples in buffered rumen fluid for 48 h using ANKOM Daisy^{II} Incubator. For all data, a completely randomized design with a 4 × 2 factorial arrangement was used. The experimental factors were straw type at 4 levels (maize, wheat, rapeseed and soybean) and fungus application at 2 levels (T.447 and control). These findings show that crude protein, ether extract, ash, organic matter and cellulose, statistically, differed (P < 0.05) from straw type and fungus application. *In vitro* ruminal dry matter digestibility (IVRDMD), *in vitro* ruminal organic matter digestibility (IVROMD) and *in vitro* ruminal digestible organic matter in dry matter (IVRDOMD) differed significantly from straw type (P < 0.001) and fungus application (P < 0.05). The crude protein and ash concentrations increased (P < 0.05) in fungal-treated straws. The ether extract, organic matter, cellulose and *in vitro* ruminal digestibility values decreased (P < 0.05) in fungal-treated samples. These results obtained showed that the fungus has been active on the straws and had some desirable effects, such as enhancement of crude protein and undesirable effects, such as decrement of ether extract, organic matter and *in vitro* ruminal digestibility.

Key words: *Trichoderma harzianum*, chemical composition, *in vitro* ruminal digestibility, straw.

INTRODUCTION

There are a lot of agricultural residues (crop residues and agro industrial residues) annually produced in agricultural countries all over the world of which Iran is no exception. The large portion of these residues are important feed stuff for ruminants and can be used as a potentially important source of carbohydrates and energy; however, the utilization of these materials as a feed source for ruminants are limited for their complex biological structure and low protein content (Rodrigues et al., 2008).

Lignocelluloses is the major component of crop residue cell wall, especially secondary cell wall, with cellulose, hemicelluloses and lignin content in which lignin inheres in the cellulose and hemicelluloses matrix. The low ability of lignocelluloses to hydrolyze (more for crystalline structure of cellulose fibrils and presence of lignin) reduces the digestibility and restricts efficient utilization of the feed produced by ruminal micro-organisms. Although, microorganisms within the rumen

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are able to exude enzymes that have potential to directly hydrolyze cellulose and hemicelluloses in the rumen. The complex network formed by cellulose, hemicelluloses and lignin reduces their digestibility because of lacking ligninolytic activity (Falcon et al., 1995; Otjen et al., 1987; Zadrazil et al., 1985). Therefore, they are not very efficient.

In addition to purely physical and chemical methods, there are some combined methods such as ammonization (Oji et al., 2007; Dean et al., 2008), urea treatment (Yalchi, 2010) alkalis and oxidant treatment (Khorvash et al., 2010a, b Granzin and Dryden, 2003), exogenous fibre degrading enzymes (Rodrigues et al., 2008; Beauchemin et al., 2003), alkali treatment-exogenous fibrolytic enzymes (Wang et al., 2004), and pressure and heat with steam (Liu et al., 1999) which have been applied successfully to break down lignocellulose chemical structure and increase the soluble carbohydrate fractions. This results in the increase of the nutritive value of agricultural residues, and improvement of the rumen digestibility and performance of ruminants.

Recently, much interest has been evinced in the use of plant cell wall degrading exogenous enzymes as direct feed supplements in ruminant diet (Gado et al., 2009; Beauchemin et al., 2003).

Available literature indicated that application of fibrolytic exogenous enzymes as additives may be efficiently useful for improving fibre digestibility and nutritive value in agricultural by-products. However, the utilization of these additives can be restricted by enzyme activities, type and dose of enzyme, enzyme application method, diet and animal physiological status (Giraldo et al., 2008; Colombatto et. al., 2003), even the level of animal productivity (Giraldo et al., 2008; Beauchemin et al, 2003), as well as the expensive expenditure of exogenous enzyme produced.

Trichoderma isolates are widely used in industrial applications (Van Wyk and Mohulatsi, 2003) as well as a common source of enzymes used in the ruminant feed as feed additives because of their high secretory capacity and inducible promoting characteristic. However, the cost of commercial purified enzymes remains very high (Von Sivers and Zacchi, 1995). In another scenario, *Trichoderma spp*. is directly introduced into substrates such as straws as feed treatment to degrade cellulose and hemicelluloses.

Our recent study indicated that *T. harzianum* isolate T.447 grew fast on wheat straw and decreased neutral detergent fiber (NDF) and increased *in vitro* digestibility more than other tested isolates from Trichoderma fungi (*T. hamatum* isolate T.614 and T.625 and *T. harzianum* isolate T.969) (Yalchi and Hajieghrari, 2010).

The purpose of this study was to examine the effect of inoculation of *T. harzianum* isolate T.447 to maize, wheat, rapeseed and soybean straws on chemical composition and *in vitro* ruminal digestibility of them.

MATERIALS AND METHODS

T. harzianum isolate T.447 (T.447) selected for this study was obtained from the collection of *Trichoderma spp*. in the Plant Pest and Disease Institute, Tehran, Iran. The isolates were grown on Potato Dextrose Agar (PDA, BDH Ltd, UK 39 g/L) medium, maintained on PDA medium and stored at 4°C for future use.

To prepare spore suspension, the isolates were re-cultured on PDA and incubated for one week at 25±1°C in dark. Five discs of mycelia agar plugs obtained from the margin of the T.447 (one-week old growing colonies) were removed with No. 3 cork borer (5 mm diameter) and placed on the surface of 100 ml PDA in a 250 ml conical flask and incubated at 25±1°C for 7 days. After the incubation period, 30 ml of double distilled water were added to each conical flask and shaken on a rotary shaker set at 80 rpm for 30 min. The concentration of T.447 spores in suspension was counted using haemocytometer which was about 10^6 to 10^7 spores per ml.

Agricultural residues containing maize (*Zea mays*), wheat (*Triticum aestivum* cultivar N80-90), rapeseed (*Brassica napus*) and soybean (*Glycine max*) straws were collected separately from several fields and were then chopped into pieces, approximately 3 cm in length and were mixed completely. A 2 kg sample was selected from each straw and then grounded in a Wiley mill (3 mm screen). Each preparation of straw was divided into two equal parts to be treated with a spore suspension of T.447 (10^6 to 10^7 spores per ml) or an equal volume of distilled water (control) in four replicates. The weight of each replicate was 220 g (200 g DM basis). The straws were inoculated with the 20 ml T.447 spore suspension or 20 ml distilled water. After spore inoculation, 300 ml of water were poured over each treatment in a tub and mixed for approximately 3 min. Then the treated samples were held in closed plastic bags and were incubated in a growth chamber at 25±1°C and 80% relative humidity for 45 days. During this period, the treatments were flicked one time every 15 days. At the end of the storage period, samples were exposed to free air and kept in shadow for 24 h to let out excessive moisture. The residual samples were oven-dried at 55°C for 48 h or until they reach to constant weight. 200 g samples of oven-dried straw from each treatment were grounded in a Wiley mill (1 mm screen) and used for chemical analysis and *in vitro* ruminal digestibility. Crude protein (CP), ether extract (EE), ash, organic matter (OM), acid detergent fiber (ADF) and acid detergent lignin (ADL) of the samples were determined by standard methods (AOAC, 1995). NDF was analyzed based on the method of Van Soest et al. (1991). Non fibrous carbohydrates (NFC), cellulose and hemicelluloses were calculated (NRC, 2001) as follows:

NFC = 100 – (CP + NDF + EE + Ash)%

Cellulose = (ADF - ADL) %

Hemicelluloses = (NDF - ADF)%

In vitro ruminal digestibility was determined using an ANKOM Daisy^{II} incubator (ANKOM Technology, Macedon, NY) by a modified method of ANKOM Technology (ANKOM, 2010a), in which determining true digestibility using neutral detergent solution after 48 h digestion was replaced with determining ash of the bags containing digestion residual. The Daisy^{II} apparatus contains four-liter digestion jars, which slowly rotate in a digestion chamber kept at a temperature of 39.5 ± 0.5°C. Two replicates of each sample were weighed (250 mg) and introduced into F57 filter bags (ANKOM Technology, Macedon, New York, USA) which had been pre-rinsed in acetone for 5 min and air-dried completely. The ANKOM bags had a pore size of 25 µm, were 55 mm long and 50 mm wide at the top and tapered to a width of 25 mm at the bottom,

Table 1. Effect of straw type and fungus application on chemical composition and *in vitro* ruminal digestibility (Probability)

Chemical composition	Effect		
	Straw	Fungus	Straw × Fungus
Crude protein	<0.001	<0.001	0.310
Ether extract	0.008	0.004	0.029
Ash	<0.001	0.005	0.412
Organic matter	<0.001	0.005	0.412
Neutral detergent fiber	<0.001	0.124	0.019
Acid detergent fiber	<0.001	0.078	0.255
Acid detergent lignin	<0.001	0.358	0.288
Cellulose	<0.001	0.019	0.207
Hemicelluloses	<0.001	0.744	0.074
NFC	<0.001	0.708	0.058
***In vitro* ruminal digestibility**			
IVRDMD	<0.001	<0.001	0.579
IVROMD	<0.001	0.007	0.491
IVRDOMD	<0.001	0.004	0.479

NFC: Non fibrous carbohydrates; IVRDMD: *In vitro* ruminal dry matter digestibility; IVROMD: *In vitro* ruminal organic matter digestibility; IVRDOMD: *In vitro* ruminal digestible organic matter in dry matter.

and made from polyester/polyethylene extruded filaments in a three-dimensional matrix to maximize the flux of solutions while minimizing particulate losses (Adesogan, 2005). The bags were heat sealed using an impulse sealer 0.5 mm from the edge of the bag. The prepared bags were placed in the jars. The jars were filled with pre-warmed (39°C) combined buffer solutions with a final pH of 6.81 (for each jar, 1330 ml of solution A: KH_2PO_4 10.0 g/L, $MgSO_4.7H_2O$ 0.5 g/L NaCl 0.5 g/L, $CaCl_2.2H_2O$ 0.1 g/L, Urea (reagent grade) 0.5 g/L and 266 ml of solution B: Na_2CO_3 15.0 g/L, $Na_2S.9H_2O$ 1.0 g/L) and placed into Daisy^{II} incubator (ANKOM, 2010a). Rumen fluid was collected from six areas of the rumen 2 h after the morning feeding from two fistulated Moghani sheep fed a diet of rolled barley (150 g/day) and alfalfa hay *ad libitum*. The ruminal fluid was inserted in a pre-warmed thermos (39°C) as well as two fistfuls of the fibrous mat from the rumen inserted in another pre-heated (39°C) thermos. Both of the thermoses were purged with CO_2 gas and were transported to the laboratory immediately. The fluid and fibrous mat were blended at a high speed for 2 min in a blender pre-warmed (39°C) and pre-purged with CO_2 gas. The blended digesta was filtered through four layers of cheesecloth and was inserted into a flask pre-warmed (39°C) and pre-purged with CO_2 gas and kept at 39°C in a CO_2 atmosphere. The 400 ml of prepared rumen fluid were introduced into each jar and purged with CO_2 gas. After 48 h of digestion in buffered rumen fluid, the bags were removed and rinsed thoroughly with cold tap water until the water was clear. Then, they were rinsed in acetone for 5 min, and were dried completely in air and oven at 100°C for 24 h. The oven-dried bags (containing digestion residual) were weighed and analyzed for concentrations of ash/organic matter (ANKOM, 2010b), and then *in vitro* ruminal dry matter digestibility (IVRDMD), *in vitro* ruminal organic matter digestibility (IVROMD) and *in vitro* ruminal digestible organic matter in dry matter (IVRDOMD) were determined.

The experimental design used in this study was a completely randomized design (CRD) by a 4 × 2 factorial arrangement. The experimental factors were straw type at four levels (maize, wheat, rapeseed and soybean straws) and fungus application at two levels (T.447 and control). There were four replicates of each treatment. The obtained data were analyzed by analysis of variance (ANOVA) procedure and the differences among treatments' means were compared by least significant difference (LSD) test at 1 and 5% significant levels with SAS software (SAS (1985) Institute Inc., Cary, NC, USA).

RESULTS

The effect of straw type and fungus application on chemical composition and *in vitro* ruminal digestibility are shown in Table 1. The obtained results indicated that CP, EE, ash, OM and cellulose significantly ($P < 0.05$) differed among straw types and fungus application treatments. In addition, obtained NDF, ADF, ADL, hemicelluloses and NFC showed significant ($P < 0.001$) differences among straw types, however, there were no differences observed regarding fungus application. Moreover, straw × fungus interaction was observed for EE and NDF ($P < 0.05$). There were substantial differences in IVRDMD, IVROMD and IVRDOMD concerning straw types ($P < 0.001$) and fungus application ($P < 0.05$) treatment, but no interaction effects were observed between them.

As shown in Table 2, the effect of straw type on chemical composition and *in vitro* ruminal digestibility indicated that there were significant ($P < 0.05$) differences in CP content; lowest CP content (42.8 g/kg) resulted from wheat straw, in contrast to the highest CP content (57.6 g/kg) obtained for maize straw. Also, CP content was 53.1 and 48.8 g/kg for rapeseed and soybean straw, respectively. The EE content ranged

Table 2. Effect of straw type on chemical composition (g/kg DM) and *in vitro* ruminal digestibility (%).

Chemical composition	Straw type				
	maize	Wheat	Rapeseed	Soybean	SEM
Crude protein	57.6[a]*	42.8[d]	53.1[b]	48.8[c]	0.46
Ether extract	12.7[a]	10.7[a]	11.4[a]	5.1[b]	0.47
Ash	59.1[c]	129.9[a]	65.2[c]	87.2[b]	0.76
Organic matter	940.9[a]	870.1[c]	934.8[a]	912.8[b]	0.76
Neutral detergent fiber	776.3[a]	777.5[a]	772.8[a]	729.8[b]	1.70
Acid detergent fiber	412.6[c]	518.3[b]	582.5[a]	581.9[a]	1.73
Acid detergent lignin	52.7[c]	83.7[b]	97.3a[b]	111.1[a]	1.63
Cellulose	360.0[c]	436.7[b]	485.2[a]	470.8[a]	1.75
Hemicelluloses	363.6[a]	259.2[b]	190.3[c]	148.0[d]	1.45
NFC	94.4[b]	39.1[c]	97.6[b]	129.2[a]	2.27
***In vitro* ruminal digestibility**					
IVRDMD	48.85[a]	40.11[c]	23.04[d]	45.62[b]	1.01
IVROMD	50.69[a]	45.36[b]	26.28[c]	45.92[b]	1.12
IVRDOMD	47.69[a]	39.46[b]	24.58[c]	41.92[b]	1.03

*Means in the same row with the different letters are significantly different ($P < 0.05$). SEM, Standard error of means; NFC, non fibrous carbohydrates; IVRDMD, *in vitro* ruminal dry matter digestibility; IVROMD, *In vitro* ruminal organic matter digestibility; IVRDOMD, *In vitro* ruminal digestible organic matter in dry matter.

from 12.7 g/kg for maize straw to 5.1 g/kg for soybean straw ($P < 0.05$). The OM content was significantly ($P < 0.05$) higher in maize and rapeseed straw samples than in soybean straw and wheat straw samples. Soybean straw had noticeably the lowest NDF and hemicelluloses ($P < 0.05$). Maize straw had the lowest ADF, ADL and cellulose content ($P < 0.05$). The NFC of soybean straw was higher than that of rapeseed, maize and wheat straw ($P < 0.05$). Maize straw had the greatest IVRDMD, IVROMD and IVRDOMD but rapeseed straw was the least for these variables ($P < 0.05$).

These findings of the effect of fungus on chemical composition and *in vitro* ruminal digestibility (Table 3) showed that CP and ash concentrations increased statistically ($P < 0.05$) in fungal-treated straws. On the other hand, The EE, OM, cellulose and *in vitro* ruminal digestibility values decreased markedly ($P < 0.05$) in fungal-treated samples.

Mean chemical composition and *in vitro* ruminal digestibility of water-treated (control) and fungal-treated maize, wheat, rapeseed and soybean straws are shown in Table 4. The CP content increased statistically ($P < 0.05$) in maize, rapeseed and soybean fungal-treated straws compared to their water-treated straws. However, CP increase occurred in the fungal-treated wheat straw compared to its water-treated straw but this difference was not significant ($P > 0.05$). The CP content in maize, wheat, rapeseed and soybean straws increased by 20.9, 9.0, 20.6, and 21.8%, respectively. Even though the EE decreased statistically ($P < 0.05$) in fungal-treated maize and rapeseed straw, this characteristic decreased and increased not significantly ($P > 0.05$) in wheat straw and soybean straw, respectively. The changes for ash (increment) and OM (decrement) characteristics were the same in all fungal-treated straws and there were no significant differences among them except for rapeseed straw. NDF and cellulose content decreased ($P < 0.05$) in fungal-treated rapeseed straw and ADF decreased ($P < 0.05$) in fungal-treated maize straw. Other parts of cell wall contents such as ADL and hemicelluloses showed no significant differences regarding straw type. NFC decreased statistically ($P < 0.05$) in fungal-treated soybean straw and this characteristic was not significantly ($P > 0.05$) different in fungal-treated of maize, wheat and rapeseed straw. The *in vitro* ruminal digestibility amounts decreased in all fungal-treated straws and this decrement was significant ($P < 0.05$) for rapeseed straw (IVROMD and IVRDOMD) and soybean straw (IVRDMD and IVRDOMD).

DISCUSSION

The low nutritive value of agricultural residues and straws has been widely demonstrated (Yalchi et al., 2010; Rodrigues et al., 2008). Straws commonly consist of cellulose and hemicellulose; they could be an excellent energy source for ruminants, except for the high lignin content and low digestibility (Beauchemin et al., 2003) that limit its value as a feed source for rumin-

Table 3. Effect of fungus on chemical composition (g/kg DM) and *in vitro* ruminal digestibility (%).

Chemical composition	Effect of fungus		
	Control	Fungus application	SEM
Crude protein	46.3[b]	54.8[a]	0.33
Ether extract	12.3[a]	7.6b	0.33
Ash	81.6[b]	89.1[a]	0.54
Organic matter	918.4[a]	910.9[b]	0.54
Neutral detergent fiber	768.4	759.8	1.20
Acid detergent fiber	528.9	518.8	1.23
Acid detergent lignin	83.8	88.6	1.15
Cellulose	445.1[a]	431.2[b]	1.24
Hemicelluloses	239.5	241.0	1.02
NFC	91.4	88.7	1.61
***In vitro* ruminal digestibility**			
IVRDMD	41.52[a]	37.29[b]	0.72
IVROMD	43.69[a]	40.42[b]	0.79
IVRDOMD	40.06[a]	36.76[b]	0.73

Means in the same row with the different letters are significantly different (P<0.05). SEM, Standard error of means; NFC, Non fibrous carbohydrates; IVRDMD, *In vitro* ruminal dry matter digestibility; IVROMD, *In vitro* ruminal organic matter digestibility; IVRDOMD, *In vitro* ruminal digestible organic matter in dry matter.

ants. Rumen microorganisms produce enzymes that have potential to directly hydrolyze the feed in the rumen, but the complex network formed by cellulose, hemicelluloses, and lignin reduces their digestibility due to lacking of ligninolytic activity (Falcon et al., 1995; Otjen et al., 1987; Zadrazil, 1985).

Therefore, it restricts efficient utilization of the feed by ruminants. A research has been undertaken to study biological delignification for improving the nutritional qualities of ruminant feeds (Zadrazil, 1997).

According to this study, there are relatively few groups of microorganisms that are able to degrade such complex compounds including fungi as well as actinomycetes and other Bacteria. However, the most efficient lignin degrading microorganisms are the white rot fungi (Akin et al., 1993). In this regards, some researches have been undertaken to study biological delignification of cell wall components of agricultural residues particularly along with cellulose and hemicelluloses by white rot fungi to improve ruminal digestibility (Jalč et al., 1997; Karunanandaa et al., 1992; Jung et al., 1992). The aerobic fungi belonging to Trichoderma genus are also able to liberate several individual enzymes to hydrolyzing complex plant carbohydrates such as cellulose, hemicelluloses and lignin to their monosaccharides or monomers constituents (Safari Sinegani et al., 2005). To our knowledge, *Trichoderma* species have the potential to colonize the substrate and hydrolyze complex plant carbohydrates making pre-digestion of the feed for upgrading feed intake in rumen digestion system. It may remain their enzymes effects in, when the inoculated feed is hoarded in rumen.

In this present study, changes of the chemical composition and *in vitro* ruminal digestibility of fungal-treated straws showed that the fungus has been active on the straws. These activities can be categorized into three classes containing (1) desirable effects, such as enhancement of CP; (2) undesirable effects, such as decrement of EE, OM and *in vitro* ruminal digestibility; and (3) effectless effects, such as tendency to increase in ADL and hemicelluloses, or tendency to decrease in NDF, ADF, and NFC. However, tendency to decrease in NDF and ADF may be classified as desirable effects and tendency to decrease in NFC may be classified as undesirable effects. The obtained results for enhancement of CP were similar to the work of Viesturs et al., (1981) which stated that *Trichoderma lignolum* treated in rice straw could be increased in the protein quantity. Whereas the CP increased and some parts of the cell wall decreased in fungal-treated straws, the *in vitro* ruminal digestibility did not improve. It seems that eupeptic sections such as NFC and EE decreased in fungal-treated straws. In other words, the fungus activities on substrates produce mainly cellulolytic and hemicellulolytic enzymes and degraded nutrients in the straws. In addition, remaining in fermentation condition for 45 days during incubation period caused some parts of the nutrients to degrade and have low digestibility values compared to those of control samples.

The data available showed no significant differences between fungal-treated wheat straw and the control

Table 4. Chemical composition (g/kg^{-1} DM) and in vitro ruminal digestibility (%) of water treated (control) and fungal treated of maize, wheat, rapeseed and soybean straw. (mean ± SE).

Chemical composition (g/kg^{-1} DM)	Maize straw			Wheat straw			Rapeseed straw			Soybean straw		
	Control	Fungal treated	Probability	Control	Fungal treated	Probability	Control	Fungal treated	Probability	Control	Fungal treated	Probability
CP	52.1 ± 1.0	63.0 ± 1.9	*	41.0 ± 1.9	44.7 ± 1.1	NS	48.1 ± 1.1	58.0 ± 2.3	*	44.0 ± 1.7	53.6 ± 3.9	*
EE	16.2 ± 3.0	9.2 ± 3.1	*	12.3 ± 0.6	9.0 ± 1.7	NS	16.9 ± 2.7	5.9 ± 1.5	*	3.93 ± 1.4	6.2 ± 1.6	NS
Ash	57.4.0 ± 2.5	60.8 ± 4.6	NS	126.0 ± 2.0	133.9 ± 2.6	NS	58.1 ± 2.8	72.3 ± 4.4	*	84.9 ± 2.8	89.5 ± 4.5	NS
OM	942.6 ± 2.5	939.2 ± 4.6	NS	874.0 ± 2.0	866.1 ± 2.6	NS	941.9 ± 2.8	927.7 ± 4.4	*	915.1 ± 2.8	910.5 ± 4.5	NS
NDF	787.3 ± 7.7	765.2 ± 6.1	NS	780.4 ± 4.1	774.7 ± 7.8	NS	786.4 ± 7.6	759.2 ± 7.9	*	719.5 ± 9.6	740.2 ± 8.7	NS
ADF	426.8 ± 11.2	398.5 ± 4.8	*	520.2 ± 8.7	516.4 ± 5.2	NS	587.7 ± 8.5	577.3 ± 7.2	NS	580.8 ± 8.6	582.9 ± 5.5	NS
ADL	55.5 ± 9.7	49.9 ± 14.8	NS	86.4 ± 4.1	77.0 ± 3.3	NS	89.5 ± 3.9	105.1 ± 6.0	NS	103.8 ± 3.0	118.4 ± 7.8	NS
CEL	371.3 ± 4.2	348.6 ± 15.1	NS	433.9 ± 6.3	439.5 ± 6.5	NS	498.2 ± 11.0	472.2 ± 2.5	*	477.0 ± 5.7	464.6 ± 2.1	NS
HEM	360.6 ± 10.1	366.7 ± 7.2	NS	260.2 ± 9.2	258.3 ± 2.6	NS	198.6 ± 4.2	181.9 ± 6.4	NS	138.7 ± 3.0	157.3 ± 4.7	NS
NFC	87.1 ± 8.7	101.8 ± 4.6	NS	40.4 ± 6.6	37.9 ± 8.3	NS	90.5 ± 8.1	104.6 ± 14.3	NS	147.7 ± 14.2	110.6 ± 11.9	*
***In vitro* ruminal digestibility**												
IVRDMD	50.67 ± 0.33	47.03 ± 2.11	NS	41.53 ± 0.61	38.70 ± 1.13	NS	24.96 ± 1.58	21.12 ± 2.20	NS	48.94 ± 0.70	42.30 ± 1.55	*
IVROMD	51.81 ± 0.44	49.56 ± 2.05	NS	45.83 ± 0.60	44.89 ± 0.94	NS	28.95 ± 1.75	23.60 ± 2.35	*	48.19 ± 0.73	43.65 ± 2.29	NS
IVRDOMD	48.84 ± 0.41	46.54 ± 1.93	NS	40.06 ± 0.52	38.87 ± 0.81	NS	27.27 ± 1.64	21.90 ± 2.18	*	44.10 ± 0.67	39.74 ± 2.08	*

SE, Standard error; Prob, Probability; NS, Not significant (P>0.05); *, Significantly difference (P<0.05). CP, Crude protein; EE, Ether extract, OM, Organic matter; NDF, Neutral detergent fiber, ADF, Acid detergent fiber; ADL, Acid detergent lignin; CEL, Cellulose; HEM, Hemicelluloses; NFC, Non fibrous carbohydrates; IVRDMD, *In vitro* ruminal dry matter digestibility; IVROMD, *In vitro* ruminal organic matter digestibility; IVRDOMD, *In vitro* ruminal digestible organic matter in dry matter.

(Table 4) for NDF content and *in vitro* ruminal digestibility values. These results are in contrast to some previous reports. Yalchi and Hajieghrari (2010) reported that NDF decreased and *in vitro* digestibility increased when wheat straw was treated by *Trichoderma harzianum* isolate T.447. Differences among studies may be related to the method of fungus application and the amount of water added.

A recent study demonstrated synergism between ruminal enzyme with exogenous enzyme in rumen indicating greater hydrolyzing activities rather than their individual activities (Morgavi et al., 2000) as well as the stimulation of rumen microbial number resulting in improving feed utilization (Nsereko et al., 2002). Moreover development of *Trichoderma* in substrate depends on several factors such as type of substrate, storage temperature, moisture content, presence of oxygen, and gaseous composition as well as other colonized microorganisms. Therefore, it seems necessary to test *Trichoderma* species and substrates to find the best situation. However, it should be noted that it is an open aspect for further studies in order to improve chemical composition and digestibility of plant material used as feed.

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Abbreviations

T.447, *Trichoderma harzianum* isolate T.447; **IVRDMD,** *in vitro* ruminal dry matter digestibility; **IVROMD,** *in vitro* ruminal organic matter digestibility; **IVRDOMD,** *in vitro* ruminal digestible organic matter in dry matter; **PDA,** potato dextrose agar; **DM,** dry matter; **CP,** crude protein; **EE,** ether extract; **OM,** organic matter; **ADF,** acid detergent fiber; **ADL,** acid detergent lignin; **NDF,** neutral detergent fiber; **NFC,** non fibrous carbohydrates.

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