

The Effect of Cellulase Enzyme Treatment on Digestibility of Rice Straw [1]

Zehra SELÇUK ¹ Mustafa SALMAN ¹ Nurcan ÇETINKAYA ¹

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¹ Ondokuz Mayıs University, Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, Kurupelit Campus, TR-55200 Atakum, Samsun - TURKEY

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Abstract

The aim of this study was to determine the effects of different levels of cellulase enzyme treatments and incubations on digestibility of rice straw. Rice straw was treated by using cellulase from *Trichoderma reesei* at 0 (Control, C), 1 (RS+CEL1), 1.5 (RS+CEL1.5) and 2% (RS+CEL2) levels (dry matter basis, DM), and treated rice straw samples were ensiled in 6 glass jars for C, RS+CEL1, RS+CEL1.5 and RS+CEL2. For control and each level of cellulase treatment, 3 glass jars were incubated at room temperature (22°C) in the dark for 30 days, 3 glass jars were incubated at 40±0.2°C in an incubator for 30 days. After the treatment, the significant increases ($P<0.05$) were determined in treatment groups compared to control for the percentage of *in vitro* true digestibility as fed (IVTD_{as fed}), *in vitro* true dry matter digestibility (IVTMD), *in vitro* true neutral detergent fiber digestibility in DM basis (IVTNDFD_{DM}) and *in vitro* true organic matter digestibility in DM basis (IVTOMD_{DM}) of rice straw. The best results obtained from the highest level of cellulase (RS+CEL2) treatment at 40°C for IVTD_{as fed}, IVTMD, IVTNDFD_{DM} and IVTOMD_{DM} were 62.99±0.22, 60.43±0.23, 30.70±0.23 and 63.58±0.34%, respectively. In conclusion, observed results showed that treatment of rice straw with cellulase improved the true digestibility.

Keywords: Fibrolytic enzyme, *In vitro* true dry matter digestibility, *In vitro* true neutral detergent fiber digestibility, Rice straw

Sellülaz Enzimi Muamelesinin Çeltik Samanı Sindirilebilirliği Üzerine Etkisi

Özet

Bu çalışmanın amacı, farklı düzeylerdeki sellülaz enzimi muamelesi ve inkübasyonunun, çeltik samanının sindirilebilirliği üzerine olan etkisini araştırmaktır. Çeltik samanı *Trichoderma reesei* kaynaklı sellülaz ile %0 (Kontrol, K), 1 (ÇS+SEL1), 1.5 (ÇS+SEL1.5) ve 2 (ÇS+SEL2) düzeylerinde (kuru maddedede, KM'de) muamele edildi. Muamele edilen çeltik samanları, kontrol ve her bir muamele grubu için 6'shar adet cam kavanoza silolandı. Kavanozların 3 tanesi karanlıkta oda sıcaklığında (22°C) diğer 3 tanesi de bir inkübatorde 40°C'de 30 gün süre ile inkübasyona bırakıldı. Çeltik samanının farklı düzeylerde sellülaz ile muamelesi sonucunda kontrol grubuna göre çeltik samanı *in vitro* gerçek sindirilebilirliğinde (IVGS_{yem}), *in vitro* gerçek kuru madde sindirilebilirliğinde (IVGKMS), kuru maddebazında *in vitro* nötral deterjan lif sindirilebilirliğinde (IVGNDFS_{KM}) ve *in vitro* organik madde sindirilebilirliğinde (IVGOMS_{KM}) önemli artışlar ($P<0.05$) saptandı. Araştırmada çeltik samanının en yüksek düzeyde sellülaz (ÇS+SEL2) ile muamele ve 40°C'de inkübe edilmesiyle IVGS_{yem}, IVGKMS, IVGNDF_{KM} ve IVGOMS_{KM} değerleri sırasıyla %62.99±0.22, 60.43±0.23, 30.70±0.23 ve 63.58±0.34 olarak bulundu. Sonuç olarak, elde edilen bulgular sellülaz enzimi ile çeltik samanı muamelesinin gerçek sindirilebilirliğini artırdığını göstermiştir.

Anahtar sözcükler: Çeltik samanı, Fibrolitik enzim, *In vitro* gerçek kuru madde sindirilebilirliği, *In vitro* gerçek nötral deterjan lif sindirilebilirliği

INTRODUCTION

In many Asian and other developing countries, cereal straws are used in ruminant nutrition because they can be easily and cheaply provided for ruminant nutrition.

Rice straw is a by-product of rice production. However, its nutritive value and digestibility are relatively low because its palatability is poor and it has high lignocellulosic complex, low crude protein value and high silica concentration resisting bacterial attachment to surface of rice straw in



İletişim (Correspondence)



+90 362 3121919/2591



zselcuk@omu.edu.tr

rumen [1]. Average dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin contents of different varieties of rice straw vary from 92.21 to 93.05%, 81.21 to 86.24%, 3.49 to 5.10%, 72.16 to 77.57%, 41.38 to 46.32% and 4.3 to 6.97%, respectively [2].

Rice straw like other straws described low quality forages may be treated with different physical (ground, pelleting/ chopping for reducing particle size, soaking, steam or X-rays treatment, cooking under pressure etc.), chemical (sodium/calcium/potassium/ammonium hydroxide, urea/ ammonia treatments etc.) physico-chemical (reducing particle size and chemical treatment, sodium hydroxide treatment and pelleting, urea treatment and pelleting, chemical treatments and steaming etc.) or biological (enzyme/white rot fungi treatment etc) methods for improving its feeding value. Although application of chemical treatments like sodium hydroxide to improve of digestibility of straw increased degradability of straw [3], it can be a cause of environmental pollution. Therefore, the interest is focused on the biological treatments due to the outgoing concerns on food safety in animal originated food.

Addition of exogenous enzymes obtained from fungi, white rot fungi and mushroom are a potential biological treatment to improve the digestibility of low quality forages because of being able to break down lignocellulose [4]. Recently, the degradation of cereal straw cell walls with fibrolytic enzymes has been reported to increase the digestibility and improve the nutritive value of cereal straws [5-7]. Beauchemin et al. [8] reports that the nutritive value of rice straw may be improved by using exogenous fibrolytic enzymes. However, use the fiber degrading enzyme as a feed additive and its treatment are important for increasing degradation of rice straw.

Harvested rice production in Turkey is around 900.000 tons [9] which is equivalent to 500.000 tons potential rice straw production capacity. Generally this high amount of rice straw has not been used efficiently for ruminant feeding in Turkey because of its low digestibility, therefore, rice straw has been ploughed, burned in the field or used for bedding of cattle. Only slight amount of rice straw has been used for ruminant nutrition mostly for buffaloes [10,11]. On the other side, there is a worldwide shortage of finding roughage for ruminant nutrition including Turkey. Hence, the aim of the study was to investigate effect of different levels of cellulase enzyme treatments on digestibility of rice straw.

MATERIAL and METHODS

Chemical analyses and *in vitro* digestibility of rice straw were conducted in the Ruminant Feed Evaluation Laboratory of Department of Animal Nutrition and

Nutritional Diseases, Faculty of Veterinary Medicine of Ondokuz Mayis University, Samsun, Turkey.

Animals

Rumen fluid was collected from three ruminally cannulated Karayaka rams, average weight 50 kg and two-year-aged (approved by Ondokuz Mayis University, the Local Ethics Committee on Animal Experiments, 18/12/2012, HADYEK 2012/70) were kept in the individually penned indoors at barn. The rams were fed twice daily (08:00 am and 17:00 pm) by 650 g of alfalfa hay and 350 g concentrate. These amounts were estimated to the level of 1.25 x maintenance requirements according to NRC [12] during the study. Fresh drinking water was freely supplied.

Chemical Analysis

Rice straws were milled for passing through a 1 mm sieve and analyzed according to AOAC methods [13]. ADF, NDF (with sodium sulfite) and acid detergent lignin (ADL) contents of rice straw used in the study were detected by Van Soest et al. [14].

Enzyme Treatments of Rice Straw

Rice straw was obtained from private dairy farm in Doganca Bafra, Samsun, Turkey. Rice straw was chopped to 5 cm length and treated with the cellulase enzyme (Farmazyme cellulase, FARMAVET International, Turkey, EC: 3.2.1.4, from *Trichoderma reseei* and activity \geq 5.000 U/kg; 1 unit of cellulase activity is described as the enzyme required to release 1 μ g reducing sugar from 4 mg/ mL sodium cellulose glycolate in one min at 37°C and pH 5.5) at the levels of 0 (C), 1 (RS+CEL1), 1.5 (RS+CEL1.5) and 2.0% (RS+CEL2) of dry matter (DM) basis of rice straw according to the method reported by Nakashima et al. [15]. The amount of water added to rice straw for enzyme treatment calculated to provide approximately 40% DM of rice straw. To ensure a homogeneous distribution of cellulase enzyme in rice straw, cellulase enzyme was dissolved in water and then was immediately sprayed on rice straw and mixed for absorption by the rice straw. After mixing, rice straw samples treated with cellulase enzyme were compressively filled into 6 glass jars each of which is a 1 L for each level of enzyme treatment. To prevent the air inlet to the jars, around of the lids of jars were coated with silicone. For each level of cellulase enzyme treatment, 3 glass jars were incubated at room temperature (22°C) in the dark for 30 days, 3 glass jars were incubated at 40 \pm 0.2°C in an incubator for 30 days. After incubation, all jars were opened and dried at 100 \pm 0.2°C in an incubator to stop the cellulase activity. Dried rice straws were ground for *in vitro* digestion technique and chemical analysis.

Preparation of Bags

The bags (Ankom F57 filter bag, Ankom Technology Corp., Fairport, NY, USA) were rinsed in acetone for 3 min

and then they were allowed to air dry. Once air dry, the bags were marked and placed in a drying oven set at 60°C for 8 h. After the bags were dried, they were kept in a desiccator before being weighed. Ground rice straw samples were weighed into the bags (triplicate per treatment) at a mass of 0.5±0.01 g per bag, sealed by an impulse bag sealer (Ankom 1915/1920 Heat Sealer, Ankom Technology Corp., Fairport, NY, USA). A bag without substrate was also used for a blank [16].

Ruminal Content Collection

Ruminal content was collected from different sites within the rumen at 3 h after morning feeding in thermos flasks and transported instantly to the laboratory. Ruminal content was strained through different layers of cheesecloth and held at 39°C under a CO₂ atmosphere.

In vitro Digestion Method

The Ankom Daisy™ *in vitro* fermentation system (Ankom Technology Corp., Fairport, NY, USA) was used for the determination of *in vitro* true digestibility, and the procedure performed was according to that described by the manufacturer [16].

In this technique, buffer solutions were prepared described by the Ankom Daisy™ *in vitro* fermentation system. An amount of 1600 mL of the buffer solution was poured into each digestion jar of incubator, before the jars were placed into the incubator with the temperature set at 39°C. The heat and agitation switches on the incubator were turned on, and the required time was allowed for the temperature of the digestion jars containing the buffer to equilibrate. Thereafter, 400 mL rumen fluid was added to each digestion jars individually. Each digestion jars were purged with CO₂ gas before activating of incubator for 48 h. After 48 h of incubation, jars were took out from their chambers, the incubation medium inside of jars was removed and bags gently washed under running cold water until they were completely clean. Thereafter, the bags were placed in the Ankom 200/220 Fiber Analyzer, and the NDF procedure was performed according to operating manual supplied by ANKOM. After the NDF procedure, the bags were dried at 105°C for 12 h and ashed in a furnace at 550°C for 6 h. The percentage of *in vitro* true digestibility as fed (IVTD_{as fed}), *in vitro* true dry matter digestibility (IVTDMD), *in vitro* true NDF digestibility in DM basis (IVTNDFD_{DM}) and *in vitro* true organic matter digestibility in DM basis (IVTOMD_{DM}) values of the samples were calculated with equations consisting the difference between the amount of incubated and the residue after NDF analysis for the different treatments [16]. Digestibilities were calculated with equations 1, 2, 3 and 4.

$$\text{IVTD}_{\text{as fed}} (\%) = 100 - [(W_3 - (W_1 \times C_1)) \times 100]/(W_2) \quad [1]$$

$$\text{IVTDMD} (\%) = 100 - [(W_3 - (W_1 \times C_1)) \times 100]/(W_2 \times \% \text{DM}_{\text{Feed}}) \quad [2]$$

$$\text{IVTNDFD}_{\text{DM}} (\%) = 100 \times [(W_2 \times \% \text{NDF}_{\text{Feed}}) - (W_3 - (W_1 \times C_1))]/(W_2 \times \% \text{DM}_{\text{Feed}}) \quad [3]$$

$$\text{IVTOMD}_{\text{DM}} (\%) = 100 - [(W_4) \times 100]/(W_2 \times \% \text{DM}_{\text{Feed}}) \quad [4]$$

where W₁ is weight of filter bag, W₂ is weight of sample, W₃ is final weight (filter bag + sample), W₄ is organic material weight (calculated after incineration of filter bags contained sample), NDF_{Feed} is % of NDF contain in feed (%DM), DM_{Feed} is % of dry matter contain in feed and C₁ is correction factor of blank filter bag value.

Statistical Analysis

Data were designed for appropriate of one-way classification with 2x4 factorial experimental design and analyzed with GLM procedure. Main effects were compared with Duncan's multiple range test. Mean differences of interaction effects were compared to Tukey test. All analyses and calculations were performed with SAS [17].

RESULTS

Dry matter, ash, crude protein, ADF, NDF and ADL contents of rice straw were 93.53, 16.68, 4.15, 41.15, 65.72 and 7.85%, respectively. The percentages of IVTD_{as fed}, IVTDMD, IVTNDFD_{DM} and IVTOMD_{DM} of rice straw untreated and treated with the different levels of cellulase enzyme, and incubated at room temperature (22°C) and 40±0.2°C for 30 d were given in Table 1 and Table 2, respectively. There were increases (P<0.05) in the percentages of IVTD_{as fed}, IVTDMD, IVTNDFD_{DM} and IVTOMD_{DM} in group RS+CEL1.5 and RS+CEL2 incubated at room temperature (22°C) for 30 d (Table 1). Although the same parameters except for the percentage of IVTOMD_{DM} increased in RS+CEL1, RS+CEL1.5 and RS+CEL2 incubated at 40°C for 30 d (Table 2). However, the percentage of IVTOMD_{DM} was the highest (P<0.05) in RS+CEL2 incubated at 40°C for 30 d. The effect of cellulase treatment and incubation temperature of rice straw on the percentages of IVTD_{as fed}, IVTDMD, IVTNDFD_{DM} and IVTOMD_{DM} of rice straw were presented in Table 3. The percentages of IVTD_{as fed}, IVTDMD, IVTNDFD_{DM} and IVTOMD_{DM} of rice straw were the highest in RS+CEL2 incubated at 40°C for 30 d (Table 3). The best results obtained from the highest level of cellulase (RS+CEL2) treatment (P<0.05) at 40°C for IVTD_{as fed}, IVTDMD, IVTNDFD_{DM} and IVTOMD_{DM} were 62.99±0.22, 60.43±0.23, 30.70±0.23 and 63.58±0.34%, respectively.

DISCUSSION

The chemical composition of rice straw except for NDF content that was lower in the present study was similar to that of Rahman et al. [2]. Yadav and Yadav [18] stated that NDF value of rice straw was 64.94% which is similar to the value of this study. The lower NDF content may be resulted in improving of fiber digestion. The ADL content of rice

Table 1. The percentages of in vitro true digestibility as fed (IVTD_{as fed}%), in vitro true dry matter digestibility (IVTDMD%), in vitro true neutral detergent fiber digestibility in dry matter basis (IVTNDFD_{DM}%) and in vitro true organic matter digestibility in dry matter basis (IVTOMD_{DM}%) of rice straw untreated and treated with cellulase enzyme + incubated at room temperature (22°C) for 30 d

Tabelo 1. Selülaz enzimi ile muamele edilen ve edilmeyen çeltik samanının oda sıcaklığında (22°C) 30 gün inkübasyonu sonucu yemin in vitro gerçek sindirilebilirlik (%IVGS_{yem}), in vitro gerçek kuru madde sindirilebilirliği (IVGKMS), kuru maddede in vitro gerçek nötral deterjan lif sindirilebilirliği (IVGNDFS_{KM}), kuru maddede in vitro gerçek organik madde sindirilebilirliği (%IVGOMS_{Km})

Digestibility, %	C X ± Sx	RS + CEL1 X ± Sx	RS + CEL1.5 X ± Sx	RS + CEL2 X ± Sx
IVTD _{as fed}	59.07±0.36b*	60.16±0.35b	61.32±0.15a	61.87±0.44a
IVTDMD	56.24±0.39b	57.40±0.37b	58.64±0.16a	59.24±0.47a
IVTNDFD _{DM}	26.51±0.39b	27.67±0.37b	28.91±0.16a	29.50±0.47a
IVTOMD _{DM}	58.92±0.30b	59.78±0.38b	61.39±0.36a	61.96±0.52a

* Different letters within the same rows indicate differences among groups (P<0.05)

C: Control, 0%; RS+CEL1: Rice Straw + cellulase treatment of dry matter basis at the level of 1%; RS+CEL1.5: Rice Straw + cellulase treatment of dry matter basis at the level of 1.5%; RS+CEL2: Rice Straw + cellulase treatment of dry matter basis at the level of 2%

Table 2. The percentages of in vitro true digestibility as fed (IVTD_{as fed}%), in vitro true dry matter digestibility (IVTDMD%), in vitro true neutral detergent fiber digestibility in dry matter basis (IVTNDFD_{DM}%) and in vitro true organic matter digestibility in dry matter basis (IVTOMD_{DM}%) of rice straw untreated and treated with cellulase enzyme + incubated at 40°C for 30 d

Tabelo 2. Selülaz enzimi ile muamele edilen ve edilmeyen çeltik samanının 40°C'de 30 gün inkübasyonu sonucu yemin in vitro gerçek sindirilebilirlik (%IVGS_{yem}), in vitro gerçek kuru madde sindirilebilirliği (IVGKMS), kuru maddede in vitro gerçek nötral deterjan lif sindirilebilirliği (IVGNDFS_{KM}), kuru maddede in vitro gerçek organik madde sindirilebilirliği (%IVGOMS_{Km})

Digestibility, %	C X ± Sx	RS + CEL1 X ± Sx	RS + CEL1.5 X ± Sx	RS + CEL2 X ± Sx
IVTD _{as fed}	58.99±0.26b*	62.25±0.23a	62.30±0.31a	62.99±0.22a
IVTDMD	56.16±0.28b	59.64±0.25a	59.70±0.33a	60.43±0.23a
IVTNDFD _{DM}	26.42±0.28b	29.90±0.25a	29.96±0.33a	30.70±0.23a
IVTOMD _{DM}	58.72±0.25c	61.90±0.23b	62.47±0.33b	63.58±0.34a

* Different letters within the same rows indicate differences among groups (P<0.05)

Table 3. The percentages of in vitro true digestibility as fed (IVTD_{as fed}%), in vitro true dry matter digestibility (IVTDMD%), in vitro true neutral detergent fiber digestibility in dry matter basis (IVTNDFD_{DM}%) and in vitro true organic matter digestibility in dry matter basis (IVTOMD_{DM}%) of rice straw untreated and treated with cellulase enzyme + incubated at room temperature (22°C) and 40°C for 30 d

Tabelo 3. Selülaz enzimi ile muamele edilen ve edilmeyen çeltik samanının oda sıcaklığında (22°C) ve 40°C'de 30 gün inkübasyonu sonucu yemin in vitro gerçek sindirilebilirlik (%IVGS_{yem}), in vitro gerçek kuru madde sindirilebilirliği (IVGKMS), kuru maddede in vitro gerçek nötral deterjan lif sindirilebilirliği (IVGNDFS_{KM}), kuru maddede in vitro gerçek organik madde sindirilebilirliği (%IVGOMS_{Km})

Treatments	IVTD _{as fed} X ± Sx	IVTDMD X ± Sx	IVTNDFD _{DM} X ± Sx	IVTOMD _{DM} X ± Sx
C inc at 22°C	59.07±0.36d*	56.24±0.39d	26.51±0.39d	58.92±0.30c
C inc at 40°C	58.99±0.26d	56.16±0.28d	26.42±0.28d	58.72±0.25c
RS+CEL1 inc at 22°C	60.16±0.35c	57.40±0.37c	27.67±0.37c	59.78±0.38c
RS+CEL1 inc at 40°C	62.25±0.23ab	59.64±0.25ab	29.90±0.25ab	61.90±0.23b
RS+CEL1.5 inc at 22°C	61.32±0.15b	58.64±0.16b	28.91±0.16b	61.39±0.36b
RS+CEL1.5 inc at 40°C	62.30±0.31ab	59.70±0.33ab	29.96±0.33ab	62.47±0.33b
RS+CEL2 inc at 22°C	61.87±0.44b	59.24±0.47b	29.50±0.47b	61.96±0.52b
RS+CEL2 inc at 40°C	62.99±0.22a	60.43±0.23a	30.70±0.23a	63.58±0.34a

* Different letters within the same column indicate differences among groups (P<0.05)

C inc at 22°C: Control incubated at 22°C; RS+CEL1 inc at 22°C: Rice Straw + cellulase treatment of dry matter basis at the level of 1% and incubated at 22°C; RS+CEL1.5 inc at 22°C: Rice Straw + cellulase treatment of dry matter basis at the level of 1.5% and incubated at 22°C; RS+CEL2 inc at 22°C: Rice Straw + cellulase treatment of dry matter basis at the level of 2% and incubated at 22°C

C inc at 40°C: Control incubated at 40°C; RS+CEL1 inc at 40°C: Rice Straw + cellulase treatment of dry matter basis at the level of 1% and incubated at 40°C; RS+CEL1.5 inc at 40°C: Rice Straw + cellulase treatment of dry matter basis at the level of 1.5% and incubated at 40°C; RS+CEL2 inc at 40°C: Rice Straw + cellulase treatment of dry matter basis at the level of 2% and incubated at 40°C

straw in the present study was lower than that of Akinfemi and Ogunwole [19] and higher than that of Rahman et al. [2]. The differences for NDF and ADL contents may be due to diversity in agro-ecological condition, variety, soil fertility, climate, and other environmental events.

Rice straw cell wall like other cell walls of plants consists of cellulose, hemicellulose and lignin. Although these components are broken down with cellulase, hemicellulase and ligninase enzymes, these enzymes except for ligninase are produced by microorganisms in the reticulorumen of ruminants [20]. Theander and Aman [21] stated that rice straw has higher leaves compared to that of other cereal straws such as barley, oats and wheat. Vadiveloo [3] reported that IVDMD of the leaves and the stems was 50-51% and 61%, respectively. Phang and Vadiveloo [22] stated that IVDMD for the leaf and stem of rice straw in goats was 56.2% and 68.5%, respectively. In the present study, IVDMD of rice straw untreated and treated with cellulase were min 56.16 ± 0.28 and max $60.43 \pm 0.23\%$. The difference in our findings may be attributed to cellulase treatment of whole rice straw including leaves and stem together.

The addition of fibrolytic enzymes in ruminant diets can increase digestibility of forages and improve productivity [23]. Wang et al. [7] reported that exogenous fibrolytic enzymes have potential effect on improving of *in vitro* fibre digestibility of barley straw. Wang et al. [7] and Salem et al. [24] mentioned that exogenous fibrolytic enzymes increase the DM and NDF digestibilities. Tang et al. [5] reported that fibrolytic enzyme supplementations improved the IVDMD and IVOMD of rice straw.

Beauchemin et al. [25] suggested that treatment of dried feeds with exogenous enzymes implemented in a liquid form was important for adsorption of enzyme to provide suitable attachment to feed material before feeding and protection against proteolytic degradation in rumen. In present study, while treatment with cellulase dissolved in water and sprayed on rice straw at the level of 1.5 and 2% and incubated at room temperature (22°C) for 30 d improved IVDMD and IVTND_{DM} (*Table 1*), IVDMD and IVTND_{DM} increased in treated groups incubated at 40°C for 30 d (*Table 2*). These results were compatible with those of Bowman et al. [26], Yang et al. [27] and Beauchemin et al. [8] who reported incorporating exogenous fibre degrading enzymes to diets improved digestion of DM and fiber in ruminants.

Treatment with the fungi for increasing feeding value of rice straw is difficult because of controlling the optimal conditions for fungal growth and when the fungi grow, they can produce some toxic metabolites. Therefore, new commercial products will have important role in future for ruminant nutrition [8]. Fazaeli et al. [28] and Rodrigues et al. [29] mentioned that enzyme treatment or its in combination with other treatments can increase the digestibility of cereal straw in ruminants. It was stated that IVDMD and

IVOMD had improved in rice straw treated with fungi [30]. In the present study, treatments of rice straw with increasing level of cellulase significantly improved IVTD_{as fed}, IVTMD, IVTNDFD_{DM} and IVTOMD_{DM} compared to untreated rice straw. This result was compatible with the statements of the some researchers [28-31]. IVTOMD_{DM} was the highest in RS+CEL2 incubated at 40°C for 30 d. This result may be attributed to the cellulase level and its activity resulted in changes in cell wall structure in incubation.

Based on our findings, it may be concluded that cellulase treated rice straw resulted in higher IVTD_{as fed}, IVTMD, IVTNDFD_{DM} and IVTOMD_{DM} values compared to untreated rice straw. The best result was obtained with the highest level of cellulase treatment (RS+CEL2) at 40°C when *in vitro* true digestibilities were considered. Although cellulase treatment improved the digestibility, further rice straw treatment experiments with exogenous enzymes alone and in combinations are required to improve digestibility.

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DECLARATION

The authors declare that they have no commercial relationship with the company.

REFERENCES

1. Widyastuti Y, Terada F, Kajikawa H, Abe A: Digestion of rice straw cell wall constituents in various rumen conditions. *Jpn Agric Res Quart*, 21, 59-64, 1987.
2. Rahman MM, Alam MR, Amin MR, Das NG: Comparative study of the nutritive values of the different varieties of rice straw. *Bang J Anim Sci*, 39, 75-82, 2010. DOI: 10.3329/bjas.v39i1-2.9679
3. Vadiveloo J: Nutritional properties of the leaf and stem of rice straw. *Anim Feed Sci Technol*, 83, 57-65, 2000. DOI: 10.1016/S0377-8401(99)00107-8
4. Jalc D: Straw enrichment for fodder production by fungi. In, Kempken F (Ed): The Mycota XI Agricultural Applications. 19-38, Springer-Verlag, Berlin, Heidelberg. 2002.
5. Tang SX, Tayo GO, Tan ZL, Sun ZH, Shen LX, Zhou CS, Xiao WJ, Ren GP, Han XF, Shen SB: Effects of yeast culture and fibrolytic enzyme supplementation on *in vitro* fermentation characteristics of low-quality cereal straws. *J Anim Sci*, 86, 1164-1172, 2008. DOI: 10.2527/jas.2007-0438
6. Yang HE, Son YS, Beauchemin KA: Effects of exogenous enzymes on ruminal fermentation and degradability of alfalfa hay and rice straw. *Asian-Aust J Anim Sci*, 24, 56-64, 2011. DOI: 10.5713/ajas.2011.90369
7. Wang Y, Ramirez-Bribiesca JE, Yanke LJ, Tsang A, McAllister TA: Effect of exogenous fibrolytic enzyme application on the microbial attachment and digestion of barley straw *in vitro*. *Asian-Aust J Anim Sci*, 25, 66-74, 2012. DOI: 10.5713/ajas.2011.11158
8. Beauchemin KA, Colombarotto D, Morgavi DP: A rationale for the development of feed enzyme products for ruminants. *Can J Anim Sci*, 84, 23-36, 2004. DOI: 10.4141/A02-103
9. Anonymous: Türkiye'de çeltik üretimi ve ithalatı, 2015. http://www.zmo.org.tr/genel/bizden_detay.php?kod=23114&tipi=17&sube=0,

Accessed: June 9, 2015.

- 10. Ozen N, Sarıcıçek BZ, Erturk MM:** Tarımsal atık ve artıkların yem olarak kullanılma olanakları. *Doğu Anadolu Tarım Kongresi*, 14-18 Eylül, Erzurum, 731-745.
- 11. Selcuk Z:** The Kızılırmak Delta: The natural habitat of Anatolian Water Buffaloes. *Kafkas Univ Vet Fak Derg*, 18, 167-168, 2012. DOI: 10.9775/kvfd.2011.5412
- 12. NRC:** Nutrient requirement of sheep. 6th ed., National Research Council, National Academy Pres, Washington, DC, USA, 1985.
- 13. AOAC:** Official Methods of Analysis, 18th ed., Association of Official Analytical Chemists, Inc., Arlington, VA, 2006.
- 14. Van Soest PJ, Robertson JD, Lewis BA:** Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J Dairy Sci*, 74, 3583-3597, 1991. DOI: 10.3168/jds.S0022-0302(91)78551-2
- 15. Nakashima Y, Ørskov ER, Adebawale EA, Ambo K:** Enzymatic manipulation of straw quality: Experience on straw upgrading. In, *Proc of the International Conference on Increasing Livestock Production through Utilization of Local Resources*, October 18-22, Beijing-China, pp.139-152, 1993.
- 16. ANKOM:** Ankcom Technology, 2005. *In vitro* true digestibility using the DAISY incubator. http://www.ankom.com/media/documents/IVMD_0805_D200.pdf, Accessed: Sep 13, 2010.
- 17. SAS:** SAS STATISTIC SOFTWARE, SAS Campus DRIVE. Cary NC, USA, 2007.
- 18. Yadav BPS, Yadav IS:** Comparative study of ammoniated wheat and paddy straws on nutrient utilization and rumen fermentation in cattle. *Indian J Anim Nutr*, 6, 215-218, 1989.
- 19. Akinfemi A, Ogunwole OA:** Chemical composition and *in vitro* digestibility of rice straw treated with *Pleurotus ostreatus*, *Pleurotus pulmonarius* and *Pleurotus tuber-regium*. *Slovak Anim Sci*, 45, 14-20, 2012.
- 20. Schiere JB, Ibrahim MNM:** Feeding of ureaammonia treated rice straw: A compilation of miscellaneous reports produced by the Straw Utilization Project (Sri Lanka). Pudoc, Wageningen, 1989.
- 21. Theander O, Aman P:** Anatomical and chemical characteristics. In, Sundstol F, Own E (Eds): *Straw and Other Fibrous By-products as Feed. Developments in Animal Veterinary Sciences*, 14. pp.45-78, Elsevier, Amsterdam, 1984.
- 22. Phang OC, Vadiveloo J:** Effects of varieties, botanical fractions and supplements of palm oil by-products on the feeding value of rice straw in goats. *Small Rumin Res*, 6, 295- 301, 1991. DOI: 10.1016/0921-4488(91)90136-E
- 23. Beauchemin KA, Holtshausen L:** Development in enzyme usage in ruminants. In, Bedfordand MR, Partridge GG (Eds): *Enzymes in Farm Animal Nutrition*. 206-230, CAB International, Oxfordshire, UK, 2010.
- 24. Salem AZM, Gado HM, Colombatto D, Elghandour MMY:** Effects of exogenous enzymes on nutrient digestibility, ruminal fermentation and growth performance in beef steers. *Livestock Science*, 154, 69-73, 2013. DOI: 10.1016/j.livsci.2013.02.014
- 25. Beauchemin KA, Colombatto D, Morgavi DP, Yang WZ, Rode LM:** Mode of action of exogenous cell wall degrading enzymes for ruminants. *Can J Anim Sci*, 84, 13-22, 2004. DOI: 10.4141/A02-102
- 26. Bowman GR, Beauchemin KA, Shelford JA:** The proportion of the diet to which fibrolytic enzyme are added affects nutrient digestion by lactating dairy cows. *J Dairy Sci*, 85, 3420-3429, 2002. DOI: 10.3168/jds.S0022-0302(02)74430-5
- 27. Yang WZ, Beauchemin KA, Rode LM:** A comparison of methods of adding fibrolytic enzymes to lactating cow diets. *J Dairy Sci*, 83, 2512-2520, 2000. DOI: 10.3168/jds.S0022-0302(00)75143-5
- 28. Fazaeli H, Azizi A, Amile M:** Nutritive value index of treated wheat straw with *Pleurotus* fungi fed to sheep. *Pak J Biol Sci*, 9, 2444-2449, 2006. DOI: 10.3923/pjbs.2006.2444.2449
- 29. Rodrigues MAM, Pinto P, Bezerra RMF, Dias AA, Guedes CVM, Gardoso VMG, Cone JW, Ferreira LMM, Colaco J, Sequeira CA:** Effect of enzyme extracts isolated from white-rot fungi on chemical composition and *in vitro* digestibility of wheat straw. *Anim Feed Sci Technol*, 141, 326-338, 2008. DOI: 10.1016/j.anifeedsci.2007.06.015
- 30. Jafari MA, Nikkhah A, Sadeghi AA, Chamani M:** The effect of *Pleurotus* spp. Fungi on chemical composition and *in vitro* digestibility of rice straw. *Pak J Biol Sci*, 10, 2460-2464, 2007.
- 31. Eun JS, Beauchemin KA, Hong SH, Bauer MW:** Exogenous enzymes added to untreated or ammoniated rice straw: Effects on *in vitro* fermentation characteristics and degradability. *Anim Feed Sci Technol*, 131, 86-101, 2006. DOI: 10.1016/j.anifeedsci.2006.01.026