Pakistan J. Zool., vol. 51(6), pp 2057-2067, 2019. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2019.51.6.2057.2067

Effects of Different Additives and Lignin Peroxidase Enzyme on *in vitro* Gas Production Kinetics and Methane Production of some Straws

Unal Kilic[1]*, Abdiwali Mohamoud Abdi[1] and Deniz Ekinci[2]

[1]*Department of Animal Science, Ondokuz Mayis University, Turkey*
[2]*Department of Agricultural Biotechnology, Ondokuz Mayis University, Turkey*

ABSTRACT

This study was aimed to determine the effects of different additives and lignin peroxidase (LiP) enzyme on the *in vitro* gas production and methane production of some straws. The study involved 8 groups for each forage, 3 different straws (wheat straw, soybean straw, sorghum straw), which comprise of 2 different LiP enzyme treatments (Available –Not Available) and 4 different treatment groups (control, 4% urea, 10% molasses and 14% urea+molasses (4% urea, 10% molasses). Hohenheim gas test was used in *in vitro* gas production measurements while infrared methane analyzer was used to determine methane production. The experiments were conducted as per the randomized factorial design in randomized complete blocks. In terms of crude protein content, the highest CP was obtained from the urea+molasses treatment for wheat, sorghum and soybean straws, while control groups were found to have the lowest CP content. The lowest NDF, ADF and lignin contents were found in sorghum straws (P<0.001). The highest gas production value was obtained from sorghum straws for 24-hours incubation process (P<0.001). Treatments did not cause any effects on 24-hours gas production for straws without enzyme addition (P>0.05). LiP enzyme was found to increase *in vitro* gas production and methane production for all straws and treatments (P<0.001). As a result, sorghum straws were proven to offer a higher feed value and additional *in vivo* and *in vitro* studies are required with the aim of determining the possibilities of using LiP enzyme in forages.

Article Information
Received 14 March 2017
Revised 11 May 2018
Accepted 10 April 2019
Available online 13 August 2019

Authors' Contribution
UK contributed in all experiment design, *in vitro* analysis of samples, acquisition of data, statistically evaluated the data and drafting of the article. AMA collected the data, did chemical analysis of samples and statistically evaluated the data. DE performed experiments and revised the article.

Key words
***In vitro* gas production, Lignin peroxidase, Methane, Straw**

INTRODUCTION

The most common issue in ruminant nutrition is the insufficiency of the forages available in winter months. As the fodder crops are not produced in sufficient amounts, manufacturers commonly have to consider straws. As straws offer low protein, energy and mineral contents, while offering higher cellulose content, their nutritive values are relatively lower (Kalkan and Filya, 2011). Commonly preferred for their cost-efficiency, straws are subjected to physical (chopping, grounding, boiling, etc.), chemical (urea and some alkali treatments) and biological methods in order to increase their nutritive values. Furthermore, it is also known that it is possible to increase the forage quality of straws using a number of additives. Scientific research has recently took an interest in biological methods (treatment with enzymes such as (cellulase, hemicellulase, pectinase and xylanase, etc.) and it is aimed to increase the nutritive value of straws with enzyme addition (Rodrigues *et al*., 2001, 2008; Hossain and Anantharaman, 2008; Kalkan and Filya, 2011; Pinto *et al*., 2012; Selcuk *et al*., 2016).

Straws offer a low digestibility with their high cellulose content and lignin content at an average between 10% and 15% (Sarnklong *et al*., 2010). It is reported that there is a direct relationship between digestibility of straw and the digestion of their lignin content (Moyson and Verachtert, 1991) and that forage digestibility is degraded in ruminants fed with poor quality forages as rumen does not have an enzyme to digest lignin. Crosby-Galváne *et al*. (2018) reported that residues of chihua pumpkin improved dry matter digestibility and reduced gas production in the *in vitro* study. In this context, use of fungi is common in order to break down the compounds including lignin (Arora *et al*., 2002; Hossain and Anantharaman, 2008; Wulandari *et al*., 2013). However, it was reported that the extended period required for the production of enzymes by fungi may lead to losses in nutrition (Ramos *et al*., 2004). Therefore, research suggests that the direct use of LiP enzyme is more practical and that LiP enzyme breaks down non-phenolic compounds including lignin (Wan and Li, 2011). Wulandari *et al*. (2013) reported that addition of ligninase enzyme in rice straw decreases the cellulose content and that 66.3% of lignin is broken down. However, literature review showed that the studies using LiP enzyme were only interested in determining the enzyme activity (Khazaal *et al*., 1990; Arora *et al*., 2002) and no studies were found in which enzyme is

* Corresponding author: unalk@omu.edu.tr
0030-9923/2019/0006-2057 $ 9.00/0

added to the rumen fluid directly. Kalkan and Filya (2011) reported that the direct addition of cellulase enzyme into forages improves the nutritive value, however, they have recommended further research on the effects of enzymes in the rumen environment. Ruminants produce approximately 16% of the methane available in the world. Even larger amounts of methane is produced especially when poor quality forages such as straws are consumed by ruminants. Therefore, consumption of straws without additives also have significant problems with ecological relevance.

This study aims to determine the effects of different additives (urea, molasses and urea+molasses) on the nutritive content, *in vitro* gas production, methane production, energy contents and organic matter digestibility of straws. Moreover, it was aimed to define the additives which will offer the best rumen conditions for straws with respect to ecology. it is known that forages have different lignocellulosic structures and that soybean straw consists of two different types of lignin (guaiacyl and syringyl) (Xu *et al*., 2007). Another purpose of this study is to determine the effects of LiP enzyme on the *in vitro* gas production and methane production of legume (soybean) and graminaes (wheat and sorghum) straws. The hypothesis of this study is LiP enzyme reduces the enteric methane production as it offers the properties suitable for the breaking down of peroxidases forming in rumen, in other words, it uses the hydrogens available in the rumen.

MATERIALS AND METHODS

Establishment of treatment groups and ensiling straws

In this study, three different straws (wheat straw, soybean straw and sorghum straw) with 4 different treatment groups (Control, urea (4%), molasses (10%) and urea + molasses (14%)) were used. Total of 12 treatment groups were established with 4 groups for each straw. The straws were ensiled into PVC laboratory silos (2.5-L capacity, 5 cm radius × 30.0 cm height) using pressing tool. A total of 45 laboratory silos (Three treatments × three straws × five replicate for each treatments) were made and stored at ambient temperature (27-34 °C).The straws included in the control group (100% straw) were silaged and preserved under laboratory conditions until other silos were opened.

As water soluble carbohydrate sources prevent lignin peroxidase (Sigma EC#1.11.1.14 = LiP) activity (Khazaal *et al*., 1993), no enzymes were added to the straw silage during the fermentation process. Moreover, Rehman *et al*. (2014) reported no significant differences for the straw silages with enzyme addition. Therefore, LiP enzyme was added in the process of *in vitro* gas production determination. In *in vitro* gas production tecnique, 2 different LiP enzyme applications (with or without) were used in the study and total of 24 treatment groups were established with 8 groups for each straw. The urea and molasses used in this study was defined in accordance with the literature review conducted (Sarwar *et al*., 2011; Rehman *et al*., 2014; Polyorach and Wanapat, 2015) . The percentages used in this study are: straw, 70%; urea 4%, molasses 10%; urea + molasses 14% and water for the remaining part.

Chemical analyses

All the silages were dried in a forced air oven at 55 °C for 72 h. Then, samples were milled in a hammer mill through a 1 mm sieve for determination of chemical compositions and *IVGP's* assays. The samples were analyzed for dry matter (DM), ash and crude protein (CP) contents were analysed according to AOAC (1998). CP was calculated by multiplying N by 6.25. The crude fiber (CF), acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) analysis were done according to the method of Van Soest *et al*. (1991) using Ankom^{A2000} automated fiber analyser. The ether extract (EE) content was determined with Ankom ^{XT15} analyzer (AOCS, 2005). The contents of cellulose (Cel=ADF-ADL), hemicellulose (HCel = NDF-ADF), organic matters (OM = DM-ash)) and nitrogen free extract (NFE=DM-(CP+ash+EE+CF)) were determined by calculation.

In order to identify the optimum LiP enzyme dosage, LiP enzyme was tried in different buffers (pH=3.5 and pH=6.0), in different dosages (0, 10µl, 100µl and 1000µ), and using hydrogen peroxide (H_2O_2) and veratryl alcohol with wheat straw and the treatments were cultured for 48 h. Then the LiP enzyme activity was read with a spectrophotometer at 310nm. One enzyme unit was defined as the amount of enzyme necessary to induce 1µmol veratryl alcohol into veratraldehite in 1 min. According to the results of preliminary study in which the remaining amount of ADL was investigated, it was found that buffer with a pH of 3.5 performs better than the buffer with a pH of 6.0 with respect to the LiP enzyme. Moreover, in spite of the positive outcomes observed at some pH=3.5 levels, it was decided to consider the pH level similar to the rumen's (5.5-7.0), therefore, as also reported by Khazaal *et al*. (1990), it was decided that the use of 1 enzyme unit, i.e. 100µl (0.1 unit), at a pH of 6.0 and a treatment without the use of veratryl alcohol and hydrogene peroxide would be suitable.

Determining in vitro *gas production of samples (Hohenheim gas test)*

Approximately 200 mg dry weight of samples were weighed into 100 ml calibrated glass syringes following

procedures of Menke and Steingass (1988). Rumen fluid used in *in vitro* gas technique collected from three Brown Swiss x Native Cattle bulls (Average 24-30 months age and 400-500 kg Live Weight) slaughtered at a slaughterhouse. The bulls fed twice daily with a diet containing alfalfa hay (55%) and barley grain (45%). Rumen fluid was brought to the laboratory within 15-20 min in thermos (38-40°C). Then, rumen fluid mixed and it was taken under CO_2 atmosphere and were filtered through two layers of cheesecloth. The syringes were warmed at 39ºC before the injection of 30 ml rumen fluid-buffer mixture (1:2) into each syringe and incubated in a water bath at 39ºC. Gas volumes were recorded at 0, 3, 6, 9, 12, 24, 48, 72 and 96 h of incubation. Five repetitions of each sample were used in *in vitro* gas production experiment. 0.1 unit LiP enzyme was added to each 1 gram of the rumen fluid-buffer mix used in *in vitro* gas production technique, while control groups were introduced only with the rumen fluid-buffer mix without enzyme addition.

Net gas productions of samples were determined at 24 h after incubation and corrected for hay standard of University of Hohenheim and blank (without enzyme and with enzyme). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) by the NEWAY computer package programme: $y = a + b (1\text{-}exp^{-ct})$ where: a, gas production from the immediately soluble fraction (ml), b, gas production from the insoluble fraction (ml), a + b, potential gas production (ml), c, gas production rate constant for the insoluble fraction (ml/h), t, incubation time (h), y, gas produced at time t. Organic matter digestibility, ME and NE_L contents of samples were estimated using equations given below:

OMD, % = 14.88+ 0.8893 GP + 0.651 ash + 0.448 CP (Menke *et al*. 1979)

ME, MJ/kg DM = 2.20+0.136GP + 0.002859 EE^2 + 0.057CP (Menke *et al*. 1979)

NE_L, MJ/kg DM = 0.101GP + 0.11 EE + 0.051CP (Menke and Steingass, 1988)

Where; GP: 24 h gas production (ml/200mg DM), EE: Ether extract (%), CP: Crude protein (%)

Determination of methane production of samples

Methane contents (%) of total gas produced at 24 h fermentation of samples were measured using a methane analyzer (Sensor Europe GmbH, Germany) according to Goel *et al*. (2008). After measuring gas produced at 24 h incubation, 25-40 mL gas samples was transferred into inlet of the methane analyzer. Methane production (mL) was calculated as follows:

Methane production (mL) = The percent of methane (%) X Total gas production (mL)

Determination of rumen fluid pH, volatile fatty acids and ammonia-N contents

The pH value of rumen fluid was determined using Hanna 1332 digital pH meter with 3 replications. The volatile fatty acids analysis of rumen fluid were done using a gas chromatography (Agilent Technologies 6890N gas chromatography, Cat. 11023, Stabilwax-DA, 30 m, 0.25 mm ID, 0.25 um df. Maximum temperature: 260°C.) according to Wiedmeier *et al*. (1987). Rumen fluid amonnia-N analysis were done using Kjeldahl methods according to Blümmel *et al*. (1997) in 3 replicates.

Statistical analysis

One Sample Kolmogorov Smirnov and normality hypothesis tests were used in order to test the compliance of the data for variance analysis and it was found that the data had a normal distribution ($P>0.05$). Levene Homogeneity of Variances test was used to test the homogeneity of the variances and it was found that the variances were homogeneous ($P>0.05$). The data obtained from the study were analysed with randomized factorial design. All analyses of the data were performed by using the SPSS Program (Windows Version of SPSS, release 20.0). Duncan's multiple range test was used for the comparison of mean values.

RESULTS

Nutrient compositions of the straws tested in the experiment are shown in Table I. According to the Table I, among all three straws the highest DM content was found in the control groups. With respect to the crude protein content, the highest CP was obtained from the urea+molasses treatment for wheat, sorghum and soybean straws ($P<0.001$). It was found that control groups offer the lowest CP content ($P<0.001$) and the treatment increases the CP content of the straws ($P<0.001$).

The highest NFE content was found in control group and the group treated with molasses ($P<0.001$). However, urea and urea+molasses addition decreased the NFE content of the straws ($P<0.001$). The lowest NDF, ADF and lignin contents were found in sorghum straws in this study ($P<0.001$). The treatments involving molasses as additive were found to have lower NDF and ADF contents. The highest NDF content was found in control group and urea treatment of wheat straw ($P<0.001$). A decrease trend in NDF content was identified for the groups treated with molasses. When compared to the control group of soybean straw, the group with urea+molasses addition was found to have a lower NDF value ($P<0.001$). The lowest ADF content was found in the urea+molasses treatment ($P<0.001$). There was no significant effect of

Table I.- Nutrient compositions of the straws used in the study (as DM%).

Samples	DM*	OM	CP	EE	CF	Ash	NFE	NDF	ADF	ADL	HCel	Cel
WSU	69.64 ± 0.91^{c}	89.01 ± 0.69^{bcd}	7.23 ± 0.14^{e}	1.35 ± 0.2^{c}	42.67 ± 0.31^{d}	10.99 ±0.69^{bcd}	37.76 ± 1.19^{d}	76.78 ± 0.61^{a}	49.44 ± 0.52^{c}	6.09 ± 0.21^{c}	27.34 ±0.32^{bc}	43.34 ± 0.72^{a}
WSM	75.32 ± 1.04^{b}	87.7 ± 0.85^{de}	4.27 ± 0.06^{g}	1.11 ± 0.23^{c}	38.34 ± 0.8^{e}	12.3 ± 0.85^{ab}	43.98 ± 0.34^{b}	71.31 ± 0.63^{b}	44.6 ± 0.55^{e}	5.63 ± 0.34^{c}	26.71 ±0.17^{bcd}	38.97 ± 0.86^{c}
WSU+M	76.55 ± 0.54^{b}	87.92 ± 0.45^{cde}	10.21 ± 0.5^{c}	1.32 ± 0.09^{c}	37.71 ± 0.36^{e}	12.08 ±0.45^{abc}	38.68 ± 0.45^{d}	69.89 ± 1.02^{b}	43.7 ± 0.29^{e}	5.48 ± 0.28^{c}	26.2 ± 1.25^{bcd}	38.22 ± 0.31^{c}
WSCont	91.81 ± 0.1^{a}	90.2 ± 0.17^{ab}	2.93 ± 0.09^{i}	1.31 ± 0.11^{c}	41.9 ± 1.36^{d}	9.8 ± 0.17^{de}	44.06 ± 1.55^{b}	78.89 ± 1.1^{a}	47.53 ± 1.92^{d}	6 ± 1.4^{c}	31.36 ± 0.82^{a}	41.53 ± 0.53^{ab}
SSU	66.22 ± 0.18^{d}	91.14 ± 0.1^{a}	10.57 ± 0.05^{e}	1.24 ± 0.19^{c}	34.44 ± 0.16^{f}	8.86 ± 0.1^{e}	44.89 ± 0.11^{b}	63.16 ± 0.22^{c}	37.49 ± 0.35^{f}	3.11 ± 0.91^{d}	25.67 ±0.45^{cd}	34.38 ± 0.98^{d}
SSM	75.99 ± 0.1^{b}	90.5 ± 0.03^{ab}	5.29 ± 0.09^{f}	3.02 ± 0.21^{a}	31.49 ± 0.8^{g}	9.5 ± 0.03^{de}	50.71 ± 0.72^{a}	59.07 ± 0.22^{d}	34.15 ± 0.28^{g}	1.96 ± 0.11^{d}	24.91 ± 0.23^{de}	32.2 ± 0.23^{d}
SSU+M	74.29 ± 0.04^{b}	91.11 ± 0.05^{a}	17.62 ± 0.05^{a}	1.6 ± 0.13^{bc}	30.67 ± 0.65^{g}	8.89 ± 0.05^{e}	41.22 ± 0.65^{c}	54.89 ± 0.42^{e}	31.48 ± 0.46^{h}	1.82 ± 0.22^{d}	23.41 ± 0.26^{e}	29.66 ± 0.66^{e}
SSCont	90.09 ± 0.63^{a}	91.33 ± 0.03^{a}	3.28 ± 0.09^{hi}	2.26 ± 0.37^{ab}	34.68 ± 0.94^{f}	8.67 ± 0.03^{e}	51.12 ± 1.24^{a}	64.7 ± 0.22^{c}	36.98 ± 0.51^{f}	2.74 ± 0.47^{d}	27.71 ± 0.29^{b}	34.24 ± 0.04^{d}
SySU	69.2 ± 0.67^{c}	87.47 ± 0.43^{e}	9.32 ± 0.07^{d}	1.6 ± 0.21^{bc}	50.2 ± 0.41^{b}	12.53 ± 0.43^{a}	26.36 ± 0.46^{f}	69.2 ± 1.24^{b}	55.35 ± 0.9^{a}	13.15 ± 0.37^{a}	13.85 ± 0.35f^{g}	42.2 ± 1.27^{a}
SySM	74.5 ± 0.87^{b}	89.36 ± 0.3b^{c}	4.66 ± 0.04^{g}	0.91 ± 0.16^{c}	50.12 ± 0.25^{b}	10.64 ± 0.3^{cd}	33.66 ± 0.59^{e}	69.21 ± 0.56^{b}	54.92 ± 0.21^{a}	12.89 ± 0.31^{ab}	14.29 ± 0.66f^{g}	42.03 ± 0.27^{a}
SySU+M	75.5 ± 0.65^{b}	86.83 ± 0.46^{e}	12.61 ± 0.26^{b}	1.53 ± 0.43^{bc}	45.36 ± 0.21^{c}	13.17 ± 0.46^{a}	27.34 ± 1.09^{f}	63.81 ± 0.58^{c}	51.13 ± 0.22^{b}	11.52 ± 0.08^{b}	12.68 ± 0.4^{g}	39.61 ± 0.29^{bc}
SySCont	90.12 ± 0.21^{a}	90.42 ± 0.26^{ab}	3.64 ± 0.01^{h}	1.41 ± 0.77^{bc}	53.1 ± 0.43^{a}	9.58 ± 0.26^{de}	32.27 ± 0.08^{e}	71.46 ± 0.28^{b}	56.48 ± 0.51^{a}	12.64 ± 0.3^{ab}	14.98 ± 0.23^{f}	43.84 ± 0.81^{a}
Significant	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

WSU, wheat straw urea; WSM, wheat straw molasses; WSU+M, wheat straw urea+molasses; WSCont, wheat straw control; SSU,sorghum straw urea; SSM, sorghum straw molasses; SSU+M, sorghum straw urea+molasses; SSCont, sorghum straw control; SySU, soybean straw urea; SySM, soybean straw molasses; SySU+M, soybean straw urea+molasses; SySCont, soybean straw control. P<0.001;a,b…, Means with different supercripts in the same column are significantly different.

urea and molasses additions to soybean straw separately on ADF content. Sorghum straw gave the lowest lignin (ADL) content among others while wheat straws followed (P<0.001). Nevertheless, there was no effect of treatments on the lignin contents of sorghum and wheat straw (P>0.05). However, it was shown that among the soybean straws, the lignin (ADL) content of urea+molasses treatment was lower than mere urea treatment (P<0.001).

The values obtained from the rumen fluid used in the *in vitro* gas production (IVGP) technique are as follows: pH, 5.35 (5.30-5.39); total volatile fatty acids content, 96.35±0.54 mmol/L ; acetic acid, 50.60±0.71 mmol/L; propionic acid, 23.69±0.21 mmol/L; butyric acid, 17.75±0.54 mmol/L; isobutyric acid, 2.12±0.25 mmol/L; valeric acid, 1.19±0.07 mmol/L; and isovaleric acid content, 1.01±0.02 mmol/L. Amonnia nitrogen content of the rumen fluid was found to be 28.85±1.05 mg/100 ml (between 221.7 and 371.0 mg/l).

Table II shows the *in vitro* gas production of the straws with and without LiP enzyme According to the table, it was found that LiP enzyme addition increases *in vitro* gas production upon each and every treatment of the straws incubated for 3 h up to 96 h. The highest gas production value was obtained from sorghum straws for 24-h incubation process (P<0.001). Wheat straw control group gave the lowest IVGP value among the straws without enzyme treatment (P<0.001). Treatments were not found to have any effects on 24-h gas production for wheat straws, sorghum straws, and soybean straws without enzyme addition (P>0.05).

Table III shows the *in vitro* gas production parameters, OMD, ME, NE_L and methane productions of straws. LiP enzyme addition increased methane production significantly for the straws (P<0.001).

Table II.- *In vitro* gas production of the straws and pH values for 96-hours incubation.

Samples/Time		3 h	6 h	9 h	12 h	24 h	48 h	72 h	96 h	96.h pH
WSU	-	4.16± 0.70^{fgh}	4.39± 0.7^{efg}	6.08± 0.95^{efg}	8.9± 0.93^{de}	22.02± 3.74^{cd}	32.02± 1.79^{def}	34.95± 1.09^{cd}	36.38± 1.26^{def}	6.41± 0.03^{bc}
	+	18.64± 0.78^{de}	21.02± 0.63^{cd}	27.58± 0.91^{bcd}	33.85± 0.9^{bc}	57.86± 1.35^{ab}	71.12± 1.35^{abc}	75.16± 2.06^{ab}	75.3± 3.02^{abc}	6.04± 0.01^{jk}
WSM	-	2.02± 0.21^{gh}	2.35± 0.39e^{fg}	4.26± 0.58^{efg}	6.73± 0.6^{de}	15.04± 0.82^{cd}	23.55± 2.07^{def}	26.67± 3.09^{df}	29.37± 2.73^{d-g}	6.42± 0.06^{bc}
	+	21.28± 0.52^{b-e}	23.53± 1.03^{a-d}	28.62± 1.83^{a-d}	34.31± 2.22^{bc}	56.8± 1.58^{ab}	70.44± 1.91^{abc}	74.63± 2.1^{ab}	76.13± 2.07^{abc}	6.05± 0.02^{jk}
WSU+M	-	1.58± 0.56^{gh}	1.35± 0.72^{fg}	2.47± 1.05^{fg}	4.73± 1.24^{de}	13.86± 1.3^{cd}	23.11± 2.03^{def}	25.02± 2.02^{df}	26.61± 1.9^{fg}	6.39± 0.01^{cd}
	+	20.73± 0.51^{cde}	23.66± 2.1^{a-d}	30.87± 3.02^{a-d}	32.47± 8.66^{bc}	55.01± 11.88^{ab}	71.01± 13.95^{abc}	76.19± 15.99^{ab}	76.42± 15.32^{abc}	6.27± 0.02^{fg}
WSCont	-	0.45± 0.21^{h}	0.67± 0.11^{g}	1.12± 0.37^{g}	2.81± 0.57^{e}	9.98± 0.78^{d}	20.62± 0.84^{f}	26± 0.82^{df}	29.02± 0.76^{efg}	6.30± 0.01^{ef}
	+	16.58± 2.48^{e}	17.78± 3.54^{d}	22.71± 4.57^{d}	31.67± 5.51^{c}	54.37± 5.17^{ab}	65.73± 9.12^{bc}	69.02± 10.72^{b}	70.96± 10.39^{bc}	6.14± 0.04^{hi}
SSU	-	2.93± 0.62^{fgh}	3.49± 0.58^{efg}	5.29± 0.62^{efg}	8.89± 0.55^{de}	16.55± 0.48^{cd}	27.24± 0.45^{def}	32.76± 1.37^{cde}	33.43± 0.34^{d-g}	6.33± 0.01^{def}
	+	22.75± 3.53^{a-d}	24.85± 4.6^{abc}	31.44± 6.03^{a-d}	38.17± 7.34^{abc}	61.83± 8.78^{ab}	80.69± 9.32^{ab}	85.63± 9.57^{a}	87.28± 9.77^{ab}	6.11± 0.02^{ijk}
SSM	-	7.17± 0.62^{f}	9.01± 0.91^{e}	12.37± 1.04^{e}	15.95± 1.14^{d}	25.55± 1.33^{c}	37.34± 1.69^{de}	42.66± 2.01^{c}	45.55± 2.04^{de}	6.32± 0.01^{def}
	+	27.64± 5.17^{a}	30.4± 7.23^{a}	36.09± 8.9^{ab}	46.06± 12.18^{a}	61.89± 13.46^{ab}	78.01± 15.29^{abc}	80.77± 14.62^{ab}	82.78± 17.21^{abc}	6.03± 0.01^{k}
SSU+M	-	2.97± 0.22^{fgh}	3.54± 0.32^{efg}	5.02± 0.39^{efg}	7.65± 0.49^{de}	14.74± 0.76^{cd}	24.67± 1.12^{def}	30.27± 1.27^{cde}	33.47± 1.45^{defg}	6.38± 0.01^{cde}
	+	26.17± 1.72^{ab}	28.9± 2.18^{ab}	36.41± 2.9^{ab}	42.78± 3.39^{abc}	65.31± 5.09^{a}	82.61± 6.54^{a}	88.29± 7.24^{a}	89.89± 7.03^{a}	6.13± 0.01^{i}
SSCont	-	6.21± 1.35^{fg}	8.09± 1.83^{ef}	11.31± 2.2^{ef}	14.53± 2.35^{d}	24.51± 3.55^{c}	37.71± 5.22^{d}	43.16± 5.12^{c}	46.27± 5.06^{d}	6.31± 0.02^{def}
	+	25.67± 3.00^{abc}	28.03± 3.74^{ab}	34.38± 4.52^{abc}	40.57± 5.58^{abc}	64.82± 4.09^{ab}	82.67± 5.4^{a}	86.08± 5.29^{a}	89.46± 5.25^{a}	5.81± 0.04^{l}
SySU	-	3.10± 0.54^{fgh}	4.23± 0.98^{efg}	5.9± 1.43^{efg}	9.6± 1.88^{de}	15.36± 2.02^{cd}	17.97± 0.7^{f}	18.79± 1.26^{df}	19.46± 1.46^{fg}	6.51± 0.01^{a}
	+	19.16± 1.20^{de}	22.3± 1.62^{bcd}	26.49± 3.33^{cd}	32.18± 3.23^{bc}	51.64± 3.52^{b}	63.62± 5.19^{c}	67.51± 5.32^{b}	67.96± 5.48^{c}	6.29± 0.02^{f}
SySM	-	3.46± 0.42^{fgh}	5.14± 0.48^{efg}	8.49± 0.57^{efg}	10.95± 0.62^{de}	15.76± 0.51^{cd}	17.32± 0.67^{f}	17.77± 0.66^{f}	18.56± 0.59^{g}	6.51± 0.02^{a}
	+	25.51± 1.00^{abc}	29.99± 1.25^{a}	36.85± 1.27^{a}	41.93± 1.07^{abc}	59.53± 2.27^{ab}	72.52± 3.4^{abc}	76.69± 3.74^{ab}	77.28± 4.08^{abc}	6.21± 0.01^{gh}
SySU+M	-	3.97± 0.30^{fgh}	6.13± 0.58^{efg}	8.86± 0.79^{efg}	13.73± 1.04^{de}	17.59± 1.3^{cd}	21.78± 1.2^{ef}	22.81± 1.13^{df}	23.49± 1.02^{fg}	6.53± 0.01^{a}
	+	21.49± 0.34^{bcd}	23.98± 0.33^{a-d}	26.69± 0.32^{cd}	33.25± 0.06^{bc}	55.4± 3.56^{ab}	65.59± 2.38^{bc}	68.53± 2.14^{b}	68.98± 2.14^{c}	6.12± 0.01^{ij}
SySCont	-	3.40± 0.50^{fgh}	5.09± 0.7^{efg}	7.7± 0.53^{efg}	10.76± 0.77^{de}	17.1± 0.77^{cd}	20.61± 0.97^{f}	21.18± 1.47^{df}	21.86± 1.88^{fg}	6.48± 0.04^{ab}
	+	23.27± 0.78^{a-d}	28.34± 1.93^{ab}	36.25± 2.89^{ab}	43.55± 2.85^{ab}	63.83± 3.35^{ab}	77.56± 3.74^{abc}	82.18± 4^{ab}	83.22± 4^{abc}	6.21± 0.03^{g}
Significant		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

For abbreviations, see Table I.

Table III.- *In vitro* gas production parameters, OMD, ME, NE_L and methane productions of straws.

Samples	LİP Enzyme	c, ml/h	a+b, ml	OMD, %	ME MJ/kg DM	NE_L MJ/kg DM	Methane, ml
WSU	-	0.037±0.007^{d-h}	39.83± 1.68^{cd}	44.86± 3.33^{c}	5.61± 0.51^{c}	2.74± 0.38^{c}	3.59± 0.53^{c}
	+	0.047± 0.003^{de}	77.61± 2.85^{ab}	76.73± 1.2^{ab}	10.49± 0.18^{ab}	6.36± 0.14^{b}	9.92± 0.14^{ab}
WSM	-	0.028± 0.005^{ghı}	31.95± 3.68^{def}	38.17±0.73^{cd}	4.49± 0.11^{cd}	1.86± 0.08^{cd}	2.45± 0.14^{c}
	+	0.043± 0.003^{def}	78.22± 2.09^{ab}	75.31± 1.4^{ab}	10.17± 0.21^{b}	6.08± 0.16^{b}	9.13± 0.48^{ab}
WSU+M	-	0.030± 0.001$^{f-ı}$	29.14± 2.06^{def}	39.65±1.15^{cd}	4.67± 0.18^{cd}	2.07± 0.13^{cd}	2.00± 0.29^{c}
	+	0.035± 0.005^{e-h}	79.7± 15.1^{ab}	76.23±10.56^{ab}	10.27± 1.62^{b}	6.22± 1.20^{b}	9.29± 2.86^{ab}
WSCont.	-	0.02± 0.001^{ı}	38.27±0.68^{cde}	31.44± 0.69^{d}	3.73± 0.1^{d}	1.30± 0.08^{d}	1.09± 0.18^{c}
	+	0.047± 0.007^{de}	72.35±10.84^{b}	70.92± 4.6^{b}	9.76± 0.7^{b}	5.78± 0.52^{b}	8.79± 2.22^{ab}
SSU	-	0.030± 0.001$^{f-ı}$	37.58±0.76^{cde}	40.1± 0.43^{cd}	5.06± 0.07^{cd}	2.35± 0.05^{cd}	2.22± 0.10^{c}
	+	0.037±0.003^{d-h}	90.43± 9.15^{a}	80.37± 7.81^{ab}	11.22± 1.19^{ab}	6.92± 0.89^{ab}	10.57± 2.29^{ab}
SSM	-	0.030± 0.001$^{f-ı}$	48.65± 2.23^{c}	46.15± 1.19^{c}	6.0± 0.18^{c}	3.18± 0.13^{c}	4.03± 0.22^{c}
	+	0.043± 0.003^{def}	84.16± 15.7^{ab}	78.47± 11.97^{ab}	10.94± 1.83^{ab}	6.85± 1.36^{ab}	11.07± 3.28^{ab}
SSU+M	-	0.020± 0.001^{ı}	39.97± 1.73^{cd}	41.67± 0.67^{cd}	5.22± 0.1^{cd}	2.56± 0.08^{cd}	2.39± 0.17^{c}
	+	0.040±0.001^{d-g}	92.34± 7.31^{a}	86.64± 4.52^{a}	12.1± 0.7^{a}	7.67± 0.51^{a}	10.43± 2.44^{ab}
SSCont.	-	0.025± 0.003^{hı}	50.42± 5.03^{c}	43.79± 3.15^{c}	5.74± 0.48^{c}	2.89± 0.36^{c}	4.02± 1.04^{c}
	+	0.04± 0.001^{d-g}	91.63± 5.14^{a}	79.64± 3.64^{ab}	11.22± 0.56^{ab}	6.96± 0.41^{ab}	11.87± 1.46^{a}
SySU	-	0.063± 0.015^{b}	19.75± 1.5^{f}	40.87± 1.8^{cd}	4.83± 0.28^{cd}	2.20± 0.20^{cd}	2.65± 0.50^{c}
	+	0.047± 0.003^{de}	69.87± 5.46^{b}	73.14± 3.14^{b}	9.76± 0.48^{b}	5.87± 0.36^{b}	7.9± 1.26^{b}
SySM	-	0.08± 0.001^{a}	18.17± 0.62^{f}	37.91± 0.45^{cd}	4.61± 0.07^{cd}	1.93± 0.05^{cd}	2.64± 0.31^{c}
	+	0.047± 0.003^{de}	78.68± 4.25^{ab}	76.84± 2.02^{ab}	10.56± 0.31^{ab}	6.35± 0.23^{b}	10.38± 0.71^{ab}
SySU+M	-	0.063± 0.003^{bc}	23.22± 1.14^{ef}	44.74± 1.16^{c}	5.32± 0.18^{cd}	2.59± 0.13^{cd}	3.07± 0.25^{c}
	+	0.045± 0.005^{de}	70.95± 2.05^{b}	78.37± 3.17^{ab}	10.46± 0.48^{ab}	6.41± 0.36^{ab}	10.08± 0.82^{ab}
SySCont.	-	0.063± 0.006^{bc}	21.98± 1.61^{ef}	37.95± 0.68^{cd}	4.74± 0.10^{cd}	2.07± 0.08^{cd}	2.59± 0.16^{c}
	+	0.05± 0.001^{cd}	84.24± 3.78^{ab}	79.52± 2.98^{ab}	11.09± 0.46^{ab}	6.79± 0.34^{ab}	11.99± 0.64^{a}
Significant		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

For abbreviations, see Table I.

While no statistically significant differences were found for the straws without enzyme addition with respect to the methane production, urea addition to soybean straw with enzyme addition proved to have a lower methane production level ($P<0.001$) and the treatments did not have significant effects on the other straws with enzyme addition ($P>0.05$). It was observed that LiP enzyme addition to straws increases the total gas production (a+b value), OMD, ME and NE_L content at a significant level for all straws and all treatments ($P<0.001$).

DISCUSSION

Chemical compositions of straws

The reason behind the decreasing DM content with ensiling process found in this study was the addition of water and molasses during the ensiling process. The reason behind the lower CP contents found for treatments with only urea when compared to treatments with molasses+urea was the added protein provided by molasses. It is known that different dosages (4%, 5% and 6% urea) of urea applied to wheat and sorghum straws increase the CP content (Kraidees, 2005; Mattoni *et al*., 2007). These findings were similar to the findings of this study and the values agree with the literature reports.

The CP content of sorghum straw was reported between 3.3% and 5.3% in a number of studies (Mattoni *et al*., 2007; Hamed and Elimam, 2009; Jonathan *et al*., 2012; ElObied and Ali, 2013) and the CP content found in this study (3.28%) was in this reported range. The CP content of soybean straw was reported between 5.0% and 7.88% in the literature (Stanton and LeValley, 2006; Mule *et al*., 2008) and the CP content found in our study (3.64%) was lower than the reported range. The CP content of wheat straw was found to be 2.93%. This content was reported by several authors (Can *et al*., 2004; Dhali *et al*., 2005; Stanton and LeValley, 2006; Hassan *et al*., 2011) at a range between 2.9% and 4.0%. Accordingly, the CP content range reported in the literature for wheat straw was close to the one found in our study. It is believed that several factors such as forage type, soil structure, fertilization, harvest time, ratio of stalks and seeds in the hay, ratio of impurities,

etc. may account for the differences between the studies. Furthermore, it was shown by El-Shatnawi and Mohawesh (2000) that CP needs of the sheep for maintenance (7-9%) and lactation (10-12%) can be compared with urea or urea+molasses treatment of the straws.

The reason behind the decreasing NDF content in straws with molasses addition is that molasses is a water soluble carbohydrate source. This fact was the reason behind the proportionately decreasing NDF content of straw. Having found to have the lowest NDF content among other straws, sorghum straws are believed to increase the feed consumption of animals when molasses addition treatments are preferred. In addition, the NDF content found in this study for sorghum straw (64.7%) was similar to the reports of Mattoni *et al*. (2007) (64.6%) and Jonathan *et al*. (2012) (69.3%). With respect to soybean straw, Fluharty (2009) reported an NDF content of 70.0% while Stanton and LeValley (2006) reported 54.0%. In this study, the NDF content of soybean straw was 71.46% which is similar to the report of Fluharty (2009). The NDF content of wheat straws was reported by a number of authors (Can *et al*., 2004; Stanton and LeValley, 2006; Fluharty, 2009) within the range between 54.4% and 73.0%. It can be seen that the value obtained in this study (78.89%) is higher than the literature reports. In a study which used 6% urea with wheat straw, it was found that the NDF content was similar to the one obtained from control group (78.8% vs. 78.3%). These results are in agreement with the findings of our study. The assessment with respect to NDF and ADF recommends the use of sorghum straws with the lowest values and urea+molasses treatment for their high digestibility and feed intake.

Treatments (urea, molasses and urea+molasses treatments) had no effect on the lignin content of neither sorghum straws nor wheat straws. The fact that the lignin content of soybean straws with urea+molasses addition was lower than the one with only urea addition may be explained with the use of molasses, a water soluble carbohydrate source during fermentation. Moreover, it may be that urea is broken down during silage fermentation, which may lead to lower lignin content in the treatment with molasses+urea addition as the urea is not extracted in the form of ammonia. The ADL content of sorghum straw was found to be 2.74% in this study; Serna-Saldivar *et al*. (2012) and Cardoso *et al*. (2013) have reported similar results (7.0% and 7.52%, respectively) while Jonathan *et al*. (2012) who stated that they have used sorghum stalks reported an ADL content of 28.2%. According to these results, the ADL data found in this study was quite lower than the literature reports. The ADL content of soybean straw was found to be 12.64%. This value is similar to the report of Maheri-Sis *et al*. (2011) (13.0%) while lower than the report of Fluharty (2009) (16.0%).The lignin content of wheat straw was found to be 6% while it was lower than the report of Fluharty (2009). Factors such as forage type, soil structure, fertilization, harvest time, ratio of stalks and seeds in the hay, treatments applied on forages before analysis (grounding size), differences in silage fermentation may account for the differences found (Kilic and Saricicek, 2006).

In vitro *gas productions and methane productions of straws*

The pH value measured from the rumen fluid remaining after 96 h incubation shows whether the buffer used is consumed by microorganisms or not in terms of *in vitro* gas production. If the pH is acidic then buffer may be consumed. In this context, it was observed that the LiP addition decreases the pH value obtained after 96 h, however, it does not acidify the environment and that it has no effect on the results. Kalkan and Filya (2011) reported that cellulose enzyme addition increases the *in vitro* gas production of wheat straw. Authors reported a 24-h gas production at 12.46 ml for the control group of wheat straw, while this value reached up to 20.19 ml with the addition of cellulase. In this study, 24-h gas production was found at 9.98 ml for the control group and the same value reached up to 54.37 ml with LiP addition. Rodrigues *et al*. (2008) reported higher gas production in groups with enzymes they have extracted from white rot fungi and stated that it improves the digestibility of forages. We have found a similar effect in this study. Nevertheless, Rodrigues *et al*. (2001) reported that the addition of enzymes to break down cell membrane in perennial rye grass silage had no effect on gas production. This result may be accounted for factors such as intrarumen conditions, processing of forages and differences in the application, dosage differences, reduced enzyme activity in the process, etc. Kalkan and Filya (2011) reported that OMD and DMD is significantly affected by the processing conditions and that there are highly positive correlations between *in vitro* gas production and OMD.

Denek *et al*. (2014) reported 24-h gas production of 38.44ml methane production of 11.31% and ME of 7.68 MJ/kg DM for wheat straw. It can be seen that the values reported in this study are lower than the ones reported by Denek *et al*. (2014). It is believed that several factors such as forage variety, soil structure, fertilization, chemical composition, harvest time, ratio of stalks and seeds in the hay and differences in the *in vitro* gas production technique used may account for the differences found (Kilic and Saricicek, 2006). Lopez *et al*. (2005) reported "a value" (59.6-50.8 ml), "c value" (0.036-0.046 ml/h) and ME values (5.66-7.38 MJ/kg DM) legume and poaceae

straws. Among these reported values, the "a value" and ME values were higher when compared to the wheat straw control and soybean straw control groups in this study. Gas production rate (c value), on the other hand, was higher than WS but lower than SS. In addition, LiP enzyme addition led to higher values in terms of a value, c value and ME values and it was suggested that LiP enzyme addition increases the forage value.

Methane, as the final product of rumen fermentation, is known to cause 8-10% energy loss. Particularly difficult to biodegrade, lignin available in straw reduces the activity of cellulose and hemicellulase enzymes provided by rumen microorganisms. Consequently, straw leaves rumen with a quite low rumen fermentation rate and cannot be utilized by ruminants which leads to methane production (Khan and Mubeen, 2012). Johnson and Johnson (1995) reported that non-fibrous carbohydrate fermentation leads to lower methane production when compared to carbohydrate fermentation of high cellulose content and noted that feeding animals with forages containing high soluble carbohydrate content will lead to reduced methane production. This is a result of the fact that poor quality forages have a lower rate of digestible parts. Accordingly, LiP addition to poor quality forages will reduce methane formation having broken down compounds with lignin, therefore increasing the nutritional value.

Dias *et al*. (2010) reported that enzyme addition increases the cellulose/lignin ration of wheat straw and Pinto *et al*. (2012) identified lignin saccharification in wheat straw exposed to lignin peoxidase enzyme. These results are in agreement with the findings of our study. Selcuk *et al*. (2016) reported that cellulase enzyme addition increases the digestibility of rice straw and Tang *et al*. (2008) reported that fibrolytic enzyme preparation (containing cellulase and xylanase) increases the cumulative gas production, IVDMD and IVOMD of poor quality forages. In this study, it was found that *in vitro* gas production and methane production increases significantly along with OMD value with the LiP enzyme use for all the straws included in the study. As it would be expected, breaking down of lignin also increases the digestibility of organic matter.

Wan and Li (2011) reported that fungal pretreatment (enzyme production) has a less significant effect in soybean straws in terms of lignoselulosic break down when compared to wheat straws and that soybean straws are more resilient to biodegradation. Wan *et al*. (2011) reported that the cellulose digestibility of soybean straw increases significantly with the pretreatment of hot water. Although soybean straw had a higher lignin content when compared to other straws experimented in this study, it was found that LiP enzyme addition increases the OMD value of all the straws.

Kalkan and Filya (2011) reported that cellulose addition increases the ME content of wheat straw. Similarly, in this study LiP enzyme addition also increased the ME content of wheat straw. Nevertheless, it is believed that the reason behind the increase in *in vitro* gas production, methane production and digestibility of straws is the fact that the lignin structure available in the forage breaks down (acetic acid production).

Kamalak (2005) reported the *in vitro* gas productions of wheat and barley straws which are rich in NDF (75.56%, 72.73% respectively) and ADF (54.33%, 53.23%, respectively) and poor in CP content (3.14%, 4.22%, respectively) are 13.50-45.33 ml and 13.33-47.00 ml, respectively for the time range between 0^{th} to 96^{th} h. In this study, a range between 0.45 and 29.02 ml was found for wheat straw for the gas productions between 0^{th} to 96^{th} h. However, these values increased up to 16.58-70.96 ml with the addition of LiP enzyme. Accordingly, it was found that LiP enzyme has an important effect on increasing *in vitro* gas production and nutritive values of wheat straw.

It was reported for the ammonia treated soybean straw that cellulase enzyme addition decreases the lignin content to 30.16% (that it increases the breaking down of lignin) (Xu *et al*., 2007). In this study, it was found that LiP enzyme addition increases the gas production and methane production which means that lignocelulosic structure is broken down. However, it is believed that the differences found accounts for the different lignocellulosic structures straws have.

CONCLUSIONS

A general assessment will show that sorghum straw offers the best forage value among other straws. In addition, it is recommended that in lands where drought is common, sorghum straw should be enriched with additives and used in animal nutrition. Sorghum straw offers a great forage potential in this respect. It was found that treatments applied in this study increased the CP content of straws when compared to control groups and that the highest crude protein contents were possible with the urea+molasses treatment. Moreover, it was shown that LiP enzyme addition increases the gas production and methane production significantly for all the treatments. When there was no enzyme addition to any of these three straws it was observed that methane production was not affected by the treatments while enzyme addition increased methane production in all straws and treatments. Nevertheless, it was found that urea addition to soybean straw led to a lower methane production when compared to the control group. Increased methane production in rumen is not a favorable situation. Feeding strategies aimed at reducing the methane production which is increased by the

use of LiP enzyme. Accordingly, it is necessary to use the energy more efficiently with urea addition to straws or its combined use with forages rich in protein. In conclusion, all the treatments applied in this experiment were proved to be useful in increasing the nutritional value of poor quality forages and straws. Thus, it will be possible to minimize the methane production caused by the use of poor quality forages. In addition to its use in animal nutrition, it is also possible to use LiP enzyme in biogas systems where methane production is desired for better efficiency of the system. However, in order to do this, the enzymes are required to be economical and to operate at an advanced level. Indeed, white-rot fungi have been studied due to their potential to produce ligninolytic enzymes for delignification of some lignocellulosic materials. In addition, it should be considered that treatment of straws with LiP enzyme before they are fed to the animals and *in vitro* and in vivo studies are recommended for the use of LiP enzyme in poor quality forages.

ACKNOWLEDGEMENTS

This work was supported by Turkish Scientific and Technological Research Council, TUBITAK (TOVAG 216O606), Turkey.

Conflict of interest statement

We declare that we have no conflict of interest.

REFERENCES

AOAC, 1998. Officinal methods of analysis. 16th Edition, AOAC International, Gaithersburg, MD.

AOCS, 2005. *Official procedure, approved procedure Am 5-04, Rapid determination of oil/fat utilizing high temperature solvent extraction. J. Am. Oil Chem. Soc.,* Urbana, IL.

Arora, D.S., Chander, M. and Gill, P.K., 2002. Involvement of lignin peroxidase, manganese peroxidase and laccase in degradation and selective ligninolysis of wheat straw. *Int. Biodeter. Biodegr.,* **50**: 115 – 120. https://doi.org/10.1016/S0964-8305(02)00064-1

Blümmel, M., Makkar, H.P.S. and Becker, K., 1997. *In vitro* gas production- a technique revisied. *J. Anim. Physiol. Anim. Nutri.,* **77**:24–34. https://doi.org/10.1111/j.1439-0396.1997.tb00734.x

Can, A., Denek, N. and Yazgan, K., 2004. Effect of urea and molasses supplementation on nutrient intake and digestibility of sheep fed with straw. *J. Anim. Vet. Adv.,* **3**: 466-469.

Cardoso, W.S., Tardin, F.D., Tavares, G.P., Queiroz, P.V., Mota, S.S. and Megumi, M.C., 2013. Use of sorghum straw (*Sorghum bicolor*) for second generation ethanol production: pretreatment and enzymatic hydrolysis. *Quim. Nova,* **36**: 623-627. https://doi.org/10.1590/S0100-40422013000500002

Crosby-Galván, M.M., Espinoza-Velasco, B. and Ramirez-Mella, M., 2018. Effect of Chihua pumpkin residue (*Cucurbita argyrosperma*) in ruminal gas production and digestibility *in vitro. Pakistan J. Zool.,* **50**: 1183-1185. http://dx.doi.org/10.17582/journal.pjz/2018.50.3.sc1

Denek, N., Avcı, M., Can, A., Daş, B., Aydın, S.S. and Savrunlu, M., 2014. The effect of different plant leaves on the in vitro methane production of Some Forages. *J. Harran Univ. Vet. Facul.,* **2**: 59-66.

Dhali, A., Mehla, R.K. and Sirohi, S.K., 2005. Effect of urea supplemented and urea treated straw based diet on milk urea concentration in crossbred Karan-Fries cows. *Italian J. Anim. Sci.,* **4**: 25-34. https://doi.org/10.4081/ijas.2005.25

Dias, A.A., Freitas, G.S., Marques, G.S., Sampaio, A., Fraga, I.S., Rodrigues, M.A., Evtugin, D.V. and Bezerra, R.M., 2010. Enzymatic saccharification of biologically pre-treated wheat straw with white-rot fungi. *Biores. Technol., **101***: 6045-6050. https://doi.org/10.1016/j.biortech.2010.02.110

ElObied, G.H. and Ali, J.A., 2013. Optimum concentrate supplement to sorghum straw to reduce live weight loss in calves during summer season in Sudan. *Int. J. agric. Sci.*, **3**: 016-021.

El-Shatnawi, M.K. and Mohawesh, Y.M., 2000. Seasonal chemical composition of saltbush in semiarid grassland of Jordan. *J. Range. Manage.,* **53**: 211–214. https://doi.org/10.2307/4003285

Fluharty, F.L., 2009. *Protein and energy supplementation of crop residues for breeding cattle.* Department Of Animal Sciences, The Ohio State University. pp. 01-05.

Goel, G., Makkar, H.P.S., Becker, K., 2008. Effect of *Sesbania sesban* and *Carduus pycnocephalus* leaves and Fenugreek (*Trigonella foenum-graecum* L.) seeds and their extract on partitioning of nutrients from roughage-and concentrate-based feeds to methane. *Anim. Feed Sci. Technol.,* **147**: 72-89. https://doi.org/10.1016/j.anifeedsci.2007.09.010

Hamed, A.H.M. and Elimam, M.E., 2009. Effects of chopping on utilization of sorghum Stover by Nubian goats. *Pakistan J. Nutri.,* 8 (10): 1567-1569. https://doi.org/10.3923/pjn.2009.1567.1569

Hassan, Z., Nisa, M., Shahzad, M.A. and Sarwar, M., 2011. Replacing concentrate with wheat straw treated with urea molasses and ensiled with manure: Effects on ruminal characteristics, in situ digestion

kinetics and nitrogen metabolism of Nili-Ravi buffalo bulls. *Asian-Australasian J. Anim. Sci.,* **24**: 1092-1099. https://doi.org/10.5713/ajas.2011.10337

Hossain, S.M. and Anantharaman, N., 2008. Effect of wheat straw powder on enhancement of ligninolytic enzyme activity using Phanerochate chrysosporium. Indian *J. Biotechnol.,* **7**: 502-507.

Johnson, K.A. and Johnson, D.E., 1995. Methane emissions from cattle. *J. Anim. Sci.,* **73**: 2483–2492. https://doi.org/10.2527/1995.7382483x

Jonathan, S.G., Okorie, A.N., Garuba, E.O. and Babayemi, O.J., 2012. Bioconversion of sorghum stalk and rice straw into value added ruminant feed using *Pleurotus pulmonarius*. *Nature Sci.,* **10**:10-16.

Kalkan, H. and Filya, I., 2011. Effects of cellulase enzyme on nutritive value, *in vitro* digestion characteristics and microbial biomass production of wheat straw. *J. Vet. Med. Kafkas Univ.,* **17**: 585-594.

Kamalak, A., 2005. Comparison of gas production parameters and metabolizable energy contents of some forages, *KSU. J. Sci. Engin.,* **8**:116-120

Khan, T.S. and Mubeen, C., 2012. Wheat straw: A pragmatic overview. *Curr. Res. J. biol. Sci.,* **4**: 673-675.

Khazaal K. A., Owen, E., Dodson, A. P., Harvey, P. and Palmer, J., 1990. A preliminary study of the treatment of barley straw with ligninase enzyme: effect on ın-vitro digestibility and chemical composition. *Biol. Wastes,* **33**: 53-62. https://doi.org/10.1016/0269-7483(90)90121-8

Khazaal, K.A., Owen, E., Dodson, A.P., Palmer, J. and Harvey, P.O., 1993. Treatment of barley straw with ligninase: effect on activity and fate of the enzyme shortly after being added to straw. *Anim. Feed Sci. Technol.,* **41**:15-21. https://doi.org/10.1016/0377-8401(93)90091-W

Kilic, U. and Sarıcicek, B.Z., 2006. Factors affecting the results of gas production technique. *J. Anim. Prod.* **47** :54-61.

Kraidees, M.S., 2005. Influence of urea treatment and soybean meal (urease) addition on the utilization of wheat straw by sheep. *Asian Australasian J. Anim. Sci.,* **18**: 957-965. https://doi.org/10.5713/ajas.2005.957

Lopez, S., Davies, D.R., Giraldez, F.J., Dhanoa, M.S., Dijkstra, J. and France, J., 2005. Assesment of nutritive value of cereal and legume straws based on chemical composition and *in vitro* digestibility. *J. Sci. Fd. Agric.,* **85**: 1550-1557. https://doi.org/10.1002/jsfa.2136

Maheri-Sis, N., Abdollahi-Ziveh, B., Salamatdoustnobar, R., Ahmadzadeh, A., Aghajanzadeh-Golshani, A. and Mohebbizadeh, M., 2011. Determining nutritive value of soybean straw for ruminants using nylon bags technique. *Pakistan J. Nutri.* **10**: 838-841.

Mattoni, M., Schiavone, A., Tarantola, M., Ladetto, G., De Meneghi, D. and Kanwe, A.B., 2007. Effect of urea treatment on the nutritive value of local sorghum and millet straw: a comparative study on growing performance of Djallonke rams. *Italian J. Anim. Sci.,* **6**: 318-320. https://doi.org/10.4081/ijas.2007.1s.318

Menke, K.H., Raab, L., Salewski, A., Steingass, H., Fritz, D. and Schneider, W., 1979. The estimation of the digestibility and metabolizable energy content of ruminant feedingstuffs from the gas production when they are incubated with rumen liquor *in vitro*. *J. agric. Sci. Camb.*, **93**:217–222. https://doi.org/10.1017/S0021859600086305

Menke, K.H. and Steingass. H., 1988. Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. *Anim. Res. Devel.,* Separate Print, **28**: 7-55.

Moyson, E. and Verachtert, H., 1991. Growth of higher fungi on wheat straw and their impact on the digestibility of the substrate. *Appl. Microbiol. Biotechnol.,* **36**: 421-424. https://doi.org/10.1007/BF00208168

Mule, R.S., Barbind, R.P., Baswade, S.V., Samale, D.T. and Adangale, S.B., 2008. Nutritive value of soybean straw in Osmanabadi kids. *Vet. World,* **1**: 314-316.

Ørskov, E.R. and McDonald, I., 1979. The estimation of protein degradability in the rumen from incubation measurements weighted according to rate of passage. *J. agric. Sci. Camb.* **92**: 499–503. https://doi.org/10.1017/S0021859600063048

Pinto, P.A., Dias, A.A., Fraga, I., Marques, G., Rodrigues, M.A., Colaço, J., Sampaio, A., Bezerra, R.M., 2012. Influence of ligninolytic enzymes on straw saccharification during fungal pretreatment. *Biores. Technol.,* **111**: 261-267. https://doi.org/10.1016/j.biortech.2012.02.068

Polyorach, S. and Wanapat, M., 2015. Improving the quality of rice straw by urea and calcium hydroxide on rumen ecology, microbial protein synthesis in beef cattle. *J. Anim. Physiol. Anim. Nutri.,* **99**: 449-456. https://doi.org/10.1111/jpn.12253

Ramos, J., Rojas, T., Navarro, F., Davalos, F., Sanjuan, RN. and Rutiaga, J., 2004. Enzymatic and fungal treatments on sugarcane bagasse for the production of mechanical pulps. *J. Agric. Fd. Chem.* **52**: 5057–62. https://doi.org/10.1021/jf030728+

Rehman, A.U., Nisa, M.U., Shazad, A., Sarwar, M., Khan, O.A. and Sharif, M., 2014. Chemical composition and digestion kinetics of urea-molasses treated wheat straw ensiled with fibrolytic enzyme in ruminally cannulated buffalo bulls. *J. Anim. Pl. Sci.,* **24**: 36-39.

Rodrigues, M.A.M., Cone, J.W., Sequeira, C.A. and Mascarenhas-Ferreira, A., 2001. Effect of the addition of cell wall degrading enzymes on fermentation kinetics of perennial ryegrass silage. *J. agric. Sci.,* **136**: 443-449. https://doi.org/10.1017/S0021859601008954

Rodrigues, M.A.M., Pinto, P., Bezerra, R.M.F., Dias, A.A., Guedes, C.V.M., Cardoso, V.M.G., Cone, J.W., Ferreira, L.M.M., Colaco, J. and Sequeira, C.A., 2008. 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. https://doi.org/10.1016/j.anifeedsci.2007.06.015

Sarnklong, C., Cone, J.W., Pellikaan, W. and Hendriks, H.W., 2010. Utilization of rice straw and different treatment to improve its feed value for ruminants: A review. *Asian-Aust. J. Anim. Sci.,* **23**: 680-692. https://doi.org/10.5713/ajas.2010.80619

Sarwar, M., Shahzad, M.A., Nisa, M.U., Afzal, D., Sharif, M. and Saddiqi, H.A., 2011. Feeding value of urea molasses-treated wheat straw ensiled with fresh cattle manure for growing crossbred cattle calves. *Trop. Anim. Hlth. Produc.,* **43**: 543-548. https://doi.org/10.1007/s11250-010-9745-5

Selcuk, Z., Salman, M. and Cetinkaya, N., 2016. The Effect of Cellulase Enzyme Treatment on Digestibility of Rice Straw. *J. Kafkas Univ. Vet. Facul.,* **22**: 43-48.

Serna-Saldívar, S.O., Chuck-Hernández, C., Pérez-Carrillo, E. and Heredia-Olea, E., 2012. *Sorghum as a multifunctional crop for the production of fuel ethanol: Current status and future trends, bioethanol*. Prof. Marco Aurelio Pinheiro Lima (Ed.), ISBN: 978-953-51-0008-9, InTech.

Stanton, T.L. and LeValley, S., 2006. *Feed composition for cattle and sheep.* Colorado State University extension, pp. 01-06.

Tang, S. X., Tayo, G. O., Tan, Z. L., Sun, Z. H., Shen, L. X., Zhou, C. S., Xiao, W.J.,Ren, G.P., Han, X.F. and Shen, S.B., 2008. Effects of yeast culture and fibrolytic enzyme supplementation on *in vitro* fermentation characteristics of low-quality cereal straws. *J. Anim. Sci.,* **86**:1164-1172. https://doi.org/10.2527/jas.2007-0438

Van Soest, P.V., Robertson, J.B. and Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.,* **74**: 3583-3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Wan, C. and Li, Y., 2011. Effectiveness of microbial pretreatment by Ceriporiopsis subvermispora on different biomass feedstocks. *Bioresour. Technol.,* **102**: 7507-7512. https://doi.org/10.1016/j.biortech.2011.05.026

Wan, C., Zhou, Y. and Li, Y., 2011. Liquid hot water and alkaline pretreatment of soybean straw for improving cellulose digestibility. *Bioresour. Technol.,* **102**: 6254-6259. https://doi.org/10.1016/j.biortech.2011.02.075

Wiedmeier, R.D., Arambel, M.J. and Walters, J.L., 1987. Effect of yeast culture and *Aspergillus oryzae* fermentation extract on ruminal characteristics and nutrient digestibility. *J. Dairy Sci.,* **70**: 2063-2068. https://doi.org/10.3168/jds.S0022-0302(87)80254-0

Wulandari, A. P., Triyana, T., Andayaningsih, P., 2013. Delignification of rice straw with ligninase from novel *Penicillium sp*. strain apw tt2 for Biopulping. *Int. J. Biosci. Biochem. Bioinform.,* **3**: 43-46. https://doi.org/10.7763/IJBBB.2013.V3.160

Xu, Z., Wang Q., Jiang, Z., Yangn, X. and Ji, Y., 2007. Enzymatic hydrolysis of pretreated soybean straw. *Biomass Bioener.,* **31**: 162–167. https://doi.org/10.1016/j.biombioe.2006.06.015