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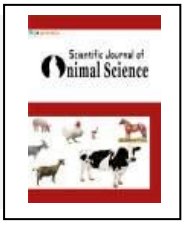

Original article

Effect of biological treatment on chemical composition and *in situ* ruminal degradability of Soybean and Canola straw in Sheep

M.Nasehi*, N.M. Torbatinejad, S. Zerehdaran, A.R. Safaei

Department of Ruminant and Poultry nutrition, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

*Corresponding author; MSc graduate, Department of Ruminant and Poultry Nutrition, Faculty of Animal Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

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ABSTRACT

This study was conducted in order to investigate the effect of biological treatment with *Pleurotus florida* fungi on chemical composition and rumen dry matter (DM) and organic matter (OM) degradability of Soybean and Canola straw. Fungi cultivation significantly decreased the amount of DM in treatments (P<0.05). Ash and crude protein (CP) content significantly increased with processing by fungi in treatments (P<0.01). The amount of Neutral detergent fiber (NDF) significantly decreased by fungi cultivation (P<0.05). Ether extract (EE), Acid detergent fiber (ADF) and Acid detergent lignin (ADL) content of treatment did not significant difference with processing by fungi. The soluble fraction (a) and potential degradability (a+b) of DM degradability significantly increased with processing in treatments (P<0.01). The insoluble but potentially degradable fraction (b) of DM degradability significantly increased with processing by fungi in Soybean straw (P<0.01) but did not significant difference in Canola straw. The (a) fraction of Canola straw OM degradability significantly increased by processing (P<0.05) but did not significant difference in Soybean straw. The (b) fraction of Soybean straw OM degradability significantly increased by processing (P<0.05) but did not significant difference in Canola straw. The (a+b) fraction of OM degradability significantly increased with processing in treatments (P<0.05). This research results generally reflect improvement in nutritive value of Soybean and Canola straw with

treatment by fungi in ruminant nutrition.

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1. 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; Yalchi and Hajieghrari., 2011). 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 microorganisms. Although, microorganisms within the rumen 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 order to break down the lignocellulosic bond of straw. The various methods that could increase its nutritive value, physical and chemical processing have been studied (Matsuzaki et al., 1994; Rahal et al., 1997). Although these methods have advantages, they are costly, low in effectiveness, not environmentally friendly and also require application of technology (Leng, 1991; Sharma et al., 1993). These factors limit their application, particularly at small farm levels. Recently, biological delignification of straw by solidstate fermentation (SSF) has been considered because of its capacity to remove lignin preferentially (Moyson and Verachtert, 1991). Fungal treatment could be an approach to convert low quality crop residues into a higher quality of ruminant feed (Arora et al., 1994; Zadrazil et al., 1997). Attempts had been made to identify species of white-rot fungi for their ability to grow on straws that improved their nutritive value (Yamakawa et al., 1992). During the SSF of wheat straw by fungi, its OM and detergent fibre content could be reduced and the lignin selectively removed from the lignocellulosic complex (Singh et al., 1990; Kundu, 1994). The CP and Ash were also increased in the treated straw (Moyson and Verachtert, 1991). Such changes were dependent on the strain of fungi and the cultural conditions (Tripathi and Yadav, 1991). Among the edible white-rot fungi, the *Pleurotus* species have been shown to be more efficient (Zadrazil et al., 1996). The potential of some species of *Pleurotus* fungi such as *P.ostreatus* and *P. eryngii* to reduce indigestible cell wall components and increase dry mater digestibility (DMD) of straw has been reported (Agosin et al., 1986; Singh et al., 1990). Therefor this study conducted in order to investigate the effect of biological treatment with *Pleurotus florida* fungi on chemical composition and rumen DM and OM degradability of Soybean and Canola straw.

2. Materials and methods

2.1. Treatment of straws and chemical analyze

Soybean and Canola straw were collected from local farms in Gorgan, Golestan province, Iran. *Pleurotus florida* fungi were used for samples treatment. Soybean and Canola straw treatment was carried out in 1000 ml bottles. 50 g of each straw was put in individual bottle and water added to give moisture content of about 85%. The bottles autoclaved at 121°C for 20 min. Each bottle was inoculated with 5% (w/w) spawns of *Pleurotus florida* fungi (Jahromi et al, 2010). Each treatment was four replicate. The bottles incubated in a incubator where temperature was automatically adjusted to 25°C and relative humidity was kept at 78±5%. After 21 days samples were dried in oven (60^0c) in order to stop fungi growth and then chemical composition, rumen DM and OM degradability were determined.

DM determined by drying the samples at 105°C overnight and Ash by igniting the samples in muffle furnace at 550°C for 8 h. EE of the samples were determined by soxhlet extraction method (AOAC, 2005). NDF, ADF and

ADL content was measured by Fiber-Tec system (Vansoest et al, 1991). Nitrogen (N) content was measured by the Kjeldahl method and CP was calculated as N*6.25(AOAC, 2005).

2.2. In situ degradation procedures

Two ruminally cannulated Dalagh rams (about 45 kg BW) were used to determine *in situ* degradation characteristics. Rams were housed in individual tie stalls bedded with sawdust. Rams fed diets containing wheat straw (70%) and concentrate mixture (30%) at the maintenance levels (NRC, 1985). The concentrate mixture contained barley grain and wheat bran.

Dacron bags (40-45 micron pore size) were filled with 3 g dried and ground samples then incubated in the rumen of rams for the periods of 0, 4, 8, 12, 24, 48, 72 and 96h. Bags were removed, washed and dried according to the procedure of Ørskov et al. (1980). Rumen degradation kinetics of DM and OM was fitted by the nonlinear model proposed by Ørskov and McDonald (1979) using Fitcurve software version 6 (Chen, 1995).

$P = a + b (1-e^{-ct})$

Where:

P = Percentage of degradability for response variables at t.

t = Time relative to incubation (h)

a = soluble fraction (%)

b = insoluble but potentially degradable fraction (%)

c = Rate constant for degradation (h^{-1})

e = 2.7182 (Natural logarithm base)

Following determination of these parameters, the effective degradability of DM and OM in the samples was calculated using and equation described by Ørskov and McDonald (1979):

$ED = a + (bc)/(c+k)$

Where:

ED = Effective degradability for response variables (%)

a = soluble fraction (%)

b = insoluble but potentially degradable fraction (%)

c = Rate constant for degradation (h^{-1})

k = Rate constant of passage (h^{-1})

Statistical Analysis

For all data, a completely randomized design with a 2 × 2 factorial arrangement were used. The experimental factors were straw type at 2 levels (Soybean and Canola straw) and fungus application at 2 levels (control and *Pleurotus florida* fungi). Each parameter was measured with four replicate. the obtained data were analyzed for parametric statistics, including analysis of variance using the general linear model procedure (GLM) and the differences among treatments' means were compared by Tukey's test. SAS statistical software (version 9.1) were used for statistical analyzes.

Tabale 1

The effect of processing on chemical compositions (%) of experimental treatments.

Items	USS	TSS	UCS	TCS	P value	SEM
DM	88^{b}	81.38^{c}	89.32^{a}	83.40^{d}	0.04	0.14
Ash	3.48^{d}	5.46^{c}	12.81^{b}	14.22^{a}	0.0001	0.33
EE	1^{a}	0.82^{a}	0.79^{a}	0.76^{a}	0.3	0.07
CP	4.36^{d}	5.37^{c}	6.63^{b}	8.48^{a}	0.009	0.12
NDF	71.42^{a}	63.83^{b}	64.89^{b}	61.02^{c}	0.01	0.61
ADF	61.87^{a}	61.69^{a}	58.73^{b}	58.02^{b}	0.7	0.75
ADL	10.22^{bc}	9.80^{c}	14.65^{a}	13.69^{ab}	0.7	0.91

USS= Untreated Soybean straw, TSS= Treated soybean straw, UCS= Untreated Canola straw, TCS= Treated Canola straw, P value= Probability value, SEM= Standard error of means; DM= Dry matter, EE= Ether extract, CP= Crude protein, NDF= Neutral detergent fiber, ADF= Acid detergent fiber, ADL= Acid detergent lignin. Means in the same row with the different superscript are significantly different {(P<0.01) and (P<0.05)}.

3. Results

3.1. Chemical composition

Chemical composition of experimental treatments is presented in Table 1. Fungal treatment significantly (P<0.05) decreased the DM content of straws, among the treatments, DM was higher for untreated canola straw (89.32%) compared to other treatments. The Ash and CP content of treatments significantly increased with fugal treatments (P<0.01), but among the treatments, CP was higher for treated Canola straw (8.48%) compared to other treatments (Table

Fungal treatment had not significant effect on EE content of experimental treatments (P>0.05) (Table 1). The NDF content of Soybean and Canola straw significantly decreased by fungal treatment (P<0.05). NDF was lower for treated Canola straw (61.02%) compared to other treatments (Table 1). ADF and ADL content of treatments did not signifant change by processing with fungi. The ADF content for treated Canola straw (58.02%) was lower compared to other treatments. The ADL content for treated Soybean straw (9.80%) was lower compared to other treatments (Table 1).

3.2. In situ degradation

The DM degradability of experimental treatments is presented in Table 2 (Fig 1). Fungal treatment significantly increased DM degradation at 96h in both straw (P<0.01). The OM degradability of experimental treatments is presented in Table 3 (Fig 2). Fungal treatment significantly increased OM degradation at 96h in both straw (P<0.01). At most of the rumen incubation periods, the degradability of DM and OM was higher for the treated Canola straw than the other treatments (Table 2 and Table 3) . Table 4 shows the degradability parameters of the DM obtained from the fitted values. The soluble fraction (a) and potential degradability (a+b) of DM degradability significantly increased with processing in treatments (P<0.01). The insoluble but potentially degradable fraction (b) of DM degradability significantly increased with processing by fungi in Soybean straw (P<0.01) but it did not significant difference in Canola straw. C fraction of DM degradability did not significant change with fungal treatment in Canola straw, but fungal treatment significantly decreased c fraction of DM degradability in Soybean straw (P<0.01). DM Effective degradability of experimental treatments significantly increased with fungal treatment in 2% out flow rate (P<0.05). Fungal treatment did not significant change at 5% out flow rate of Soybean straw DM degradability, but significantly increased the amount of DM effective degradability of Canola straw at 5% out flow rate (P<0.05).

Table 2
Ruminal Dry matter degradation (%) of treatments at different incubation times.

Incubation time (h)	SS	TSS	CS	TCS	P value	SEM
0	17.72^{c}	19.47^{b}	19.85^{b}	21.97^{a}	0.0008	0.72
4	21.97^{c}	26.42^{a}	22.72^{c}	25.05^{b}	0.0001	0.54
8	27.25^{b}	29.22^{ab}	28.72^{ab}	31.52^{a}	0.05	1.37
12	29.55^{c}	31.97^{b}	33.02^{b}	38.57^{a}	0.0001	1.09
24	39.30^{c}	36.40^{d}	41.72^{b}	44.27^{a}	0.0001	0.85
48	43.07^{b}	42.52^{b}	46.25^{b}	50.02^{a}	0.003	1.70
72	46.85^{c}	50.02^{b}	48.40^{bc}	53.05^{a}	0.0001	0.87
96	47.57^{c}	55.82^{a}	49.40^{b}	55.25^{a}	0.0001	0.73

SS= Soybean straw, TSS= Treated soybean straw, CS= Canola straw, TCS= Treated Canola straw; P value= Probability value, SEM= Standard error of means, Means in the same row with the different superscript are significantly different {(P<0.01) and (P<0.05)}.

Table 3
Ruminal Organic matter degradation (%) of treatments at different incubation times.

Incubation time (h)	SS	TSS	CS	TCS	P value	SEM
0	19.40^{c}	19.86^{c}	22.42^{a}	24.39^{a}	0.0001	0.64
4	21.94^{d}	27.19^{b}	25.04^{c}	38.65^{a}	0.0001	0.65
8	26.56^{c}	29.62^{b}	30.87^{b}	47.55^{a}	0.006	1.06
12	28.40^{c}	33.64^{b}	34.49^{b}	50.69^{a}	0.01	0.71
24	42.45^{b}	37.03^{c}	42.61^{b}	53.73^{a}	0.0001	1.15
48	44.92^{c}	43.28^{c}	47.55^{b}	55.57^{a}	0.0001	1.10
72	47.85^{c}	47.52^{c}	50.86^{b}	57.78^{a}	0.0008	0.85
96	48.72^{d}	55.27^{b}	53.44^{c}	60.50^{a}	0.0001	0.61

SS= Soybean straw, TSS= Treated soybean straw, CS= Canola straw, TCS= Treated Canola straw; P value= Probability value ,SEM= Standard error of means, Means in the same row with the different superscript are significantly different {(P<0.01)}.

DM Effective degradability of Canola straw increased with fungal treatment in 8% out flow rate (P<0.05), but fungal treatment did not significant difference in DM effective degradability of Soybean straw at 8% out flow rate. Table 5 shows the degradability parameters of the OM obtained from the fitted values. The (a) fraction of Canola straw OM degradability significantly increased by processing (P<0.05), but did not significant difference in Soybean straw.

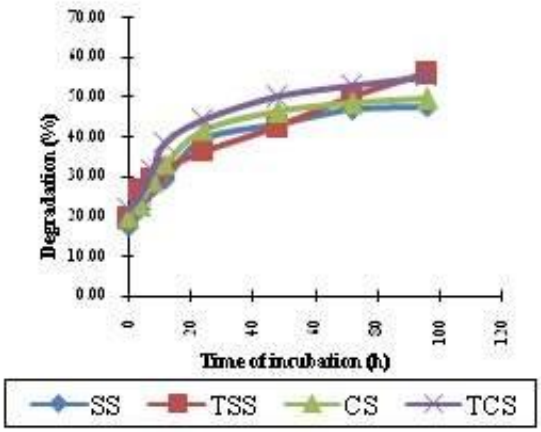

Fig. 1. DM degradation of treatments.

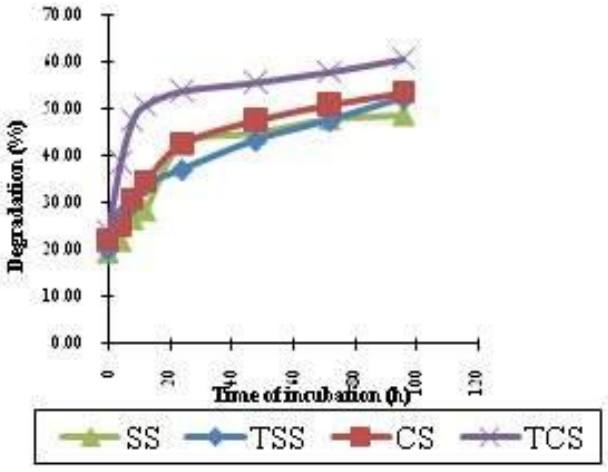

Fig. 2. OM degradation of treatments.

The (b) fraction of Soybean straw OM degradability significantly increased by processing (P<0.05), but did not significant difference in Canola straw. The (a+b) fraction of OM degradability significantly increased with processing in treatments (P<0.05).

C fraction of OM degradability did not significant change with treatment in Soybean straw, but fungal treatment significantly increased this fraction in Canola straw (P<0.01). Biological treatment with fungi significantly increased the amount of OM effective degradability at 2% out flow rate (P<0.01). OM effective degradability of Soybean straw at 5% and 8% out flow rates did not significant change with fungal treatment, but OM effective degradability of Canola straw at these out flow rates significantly increased with fungal treatment (P<0.01). (Table 5).

Table 4
Ruminal Dry matter degradation parameters and effective degradability of treatments.

Items	SS	TSS	CS	TCS	P value	SEM
a (%)	17.40^{b}	22.67^{a}	18.65^{b}	21.05^{a}	0.007	0.45
b (%)	30.72^{b}	40.05^{a}	30.70^{b}	33.32^{b}	0.007	1.04
a+b (%)	48.12^{c}	62.70^{a}	49.40^{c}	54.37^{b}	0.001	1.16
c (/h)	0.04^{a}	0.01^{b}	0.05^{a}	0.05^{a}	0.0005	0.003
ED= 0.02	38.60^{c}	40.92^{b}	40.30^{b}	44.95^{a}	0.01	0.39
ED=0.05	32^{c}	32.80^{bc}	34.35^{b}	38.82^{a}	0.01	0.49
ED=0.08	28.57^{c}	29.67^{bc}	30.80^{b}	34^{a}	0.04	0.47

SS= Soybean straw, TSS= Treated soybean straw, CS= Canola straw, TCS= Treated Canola straw; a= Soluble fraction, b= insoluble but potentially degradable fraction, a+b= potential degradability, c= rate of degradation of fraction *b* (h-1), ED= effective degradability in out flow rates (0.02, 0.05, 0.08) h-1, P value= Probability value ,SEM= Standard error of means, Means in the same row with the different superscript are significantly different {(P<0.01)}.

Table 5
Ruminal Organic matter degradation parameters and effective degradability of treatments.

Items	SS	TSS	CS	TCS	P value	SEM
a (%)	17.69^{a}	23.12^{ab}	21.75^{b}	24.66^{a}	0.03	0.53
b (%)	31.57^{b}	35.12^{a}	31.42^{b}	32.71^{ab}	0.1	0.81
a+b (%)	49.26^{c}	58.25^{a}	53.18^{b}	57.37^{a}	0.02	0.95
c (/h)	0.04^{b}	0.02^{b}	0.04^{b}	0.12^{a}	0.001	0.01
ED= 0.02	39.65^{d}	40.85^{c}	42.87^{b}	53.20^{a}	0.0001	0.35
ED=0.05	32.80^{c}	33.45^{c}	36^{b}	48.62^{a}	0.0001	0.39
ED=0.08	29.22^{c}	30.40^{c}	32.47^{b}	45.32^{a}	0.0001	0.38

SS= Soybean straw, TSS= Treated soybean straw, CS= Canola straw, TCS= Treated Canola straw; a= Soluble fraction, b= insoluble but potentially degradable fraction, a+b= potential degradability, c= rate of degradation of fraction *b* (h-1), ED= effective degradability in out flow rates (0.02, 0.05, 0.08) h-1, P value= Probability value ,SEM= Standard error of means, Means in the same row with the different superscript are significantly different {(P<0.01) and (P<0.05)}.

4. Discussion

4.1. Chemical composition

The protein content of the mycelium was reported relatively high (Ragunathan et al., 1996), so it was expected that the treated straw, that contained fungal mycelium to have a higher concentration of CP. An increase of CP content in wheat straw incubated with *Pleurotus* species had also been reported (Ardon et al., 1996; Zadrazil et al., 1996; Fazaeli et al, 2004).

The increase in the CP contents may be due to secretion of certain extra cellular enzymes which are proteineous in nature into the waste during their breakdown and its subsequent metabolism (Kadiri, 1999; Akinfemi et al, 2009). CP increase could also be due to the capture of excess nitrogen by aerobic fermentation (Sallam et al., 2007). suggesting that the treated substrates are good source of protein for livestock. This agrees with the findings of Zadrazil (1993), Belewu and Okhawere (1998), and Iyayi and Aderolu (2004). The NDF content of Soybean and Canola straw significantly decreased by fungal treatment (P<0.05) (Table 1). This was due to the natural habitats of the white-rote fungi that largely depend on organic carbon (for their energy requirement) including carbon in the form of structural material such as lignocellulosic (Jennings and Lysek, 1999). The losses of NDF from the straw suggested that these fungi could solubilize and utilize the cell wall as carbon source and thus changed the ratio of insoluble to soluble carbohydrates in the straw. The decrease in NDF contents of the treated straw has been supported by other reports (Singh, 1990; Yalchi and Hajieghrari., 2011). However, the potential of NDF degradation among species of fungi could be different (Jalc et al., 1996; Zadrazil et al., 1996).

4.2. In situ degradability

Fungal treatment significantly increased DM and OM degradation at 96h in both straw ($P<0.01$) (Table1 and Table 2). The reason for such improvement in the degradability may be related to the breaking down of cell wall bonds during the fermentation of straw with the fungi (Jennings and Lysek, 1996; Call and Mücke, 1997; Fazaeli et al, 2004). These higher values of degradability parameters in fungal treated straws may be explained by the lower cell wall components in these treatments (Table 4 and Table 5). Valmaseda et al. (1991) and Gutierrez et al. (1996) noted that fermentation of straw with *Pleurotus* fungi decreased the cell wall components and increased the soluble fraction of the carbohydrates in the straw that could be as a result of the enzymatic degradation.

5. Conclusion

In conclusion, treatment of Soybean and Canola straw with *Pleurotus florida* fungi, resulted in a reduction of those cell wall components and increasing of CP and rumen degradability. The results obtained in this study suggest that the treatment of Soybean and Canola straw by the application of fungi will help in conversion of agricultural wastes to higher quality ruminant feed thereby enhancing their digestibility by ruminants. It is therefore recommended that more work should geared towards this direction to harness the hidden potentials of agricultural wastes for the benefit of the developing countries.

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