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Effect of *Pleurotus florida* on *In vitro* Gas Production of Wheat Stubble and Date Palm Leaf

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Abstract: Availability of roughage constituents to the rumen microbes and supplying adequate nutrients intensely depends on their fibrous properties. Manipulation of these potentially feedstuffs are the great challenges to the nutritionists. Biological upgrading of crop residues is one of the approaches to enhance the release of plant nutrients in the rumen environment by the microbes. Effectiveness of *Pleurotus florida* on gas production properties of untreated and fungal treated Wheat Stubble (WS) and Date Palm Leaf (DPL) at 2, 4, 6, 8, 12, 24, 48, 72 and 96 h was investigated. Cumulative gas production from untreated WS was significantly higher than treated WS. This effect was not observed in DPL. Total gas productions, irrespective of substrate, in control, mycelial and harvesting stages were significantly different (35.83, 29.50 and 27.45 mL, respectively). Potential gas production was not affected by fungal treatment; however, it was significantly lower in DPL than in WS. Rate of gas production (c) significantly decreased at harvesting stage compare to the control in both substrates. In addition, "c" was higher in WS than in DPL. Predictive Effective Degradability (ED) of WS at different outflow rates was significantly higher than those observed for DPL. As a result of this experiment, it can be concluded that *Pleurotus florida* treatments of both WS and DPL resulted in no improvement in gas production properties of these 2 roughages.

Key words: *Pleurotus florida*, wheat stubble, date palm leaf, gas production

INTRODUCTION

Ruminants have the capacity to utilize crop residues, which generally have poor nutritional value in terms of their protein and energy. These crop residues are traditionally fed to the animals as the main part of the ration in many of the developing countries However, Dry Matter (DM) intake is not adequate to fulfill the requirements of livestock (Dixon and Egan, 2000). Limitation in DM intake is due to low rate and extent of fermentation of these residues. Date Palm Leaf (DPL) is not fed to animals because of its low palatability and digestibility (Al-Yousef *et al*., 1994; El-Din and Tag-El-Din, 1996; Bahman *et al*., 1997). However, these may be overcome by some chemical or biological treatments. During the last decades efforts have been made to improve the nutritive value of poor quality unconventional crop residues but there is no information about fungal treatment of DPL and its comparison with Wheat Stubble (WS). The possibility of biological methods of roughage treatment using white-rot-fungi (Falcon *et al*., 1995; Jalk *et al*., 1998) to improve their quality has a great appeal as an alternative to the use of corrosive and pollutant chemicals. However, it should be taken into account that organism grown on the roughage must obtain its energy from the roughage itself (Jung *et al*., 1992; Miki and Okano, 2005; Safari *et al*., 2005). Thus the present investigation was undertaken to evaluate *in vitro* gas production of untreated and fungal treated date palm leaf to that of wheat stubble, a more common roughage used in other countries.

MATERIALS AND METHODS

Wheat stubble and date palm leaf were chopped into 5-7 and 2-3 cm length, respectively. Air-dried 3 kg wheat stubble (harvested manually at ground level) or 6 kg date palm leaf were packed in nylon gunnies (100×120 cm), separately. The gunnies were then soaked in a water pool overnight; thereafter contents were transferred into 45×90 cm plastic bags and pasteurized at 80°C in a boiling pot for 1.5 h (Adamovic *et al*., 1998; Fazaeli *et al*., 2003). All the bags were removed from boiling pot, suspended in fermentation houses and left overnight to drain the excess moisture and then cooled.

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The pasteurized wheat stubble and date palm leaf were removed from the plastic bags, inoculated with *Pleurotus florida* spawn at a rate of 3-5% (w/w) and re-packed into plastic bags (Zadrazil *et al*., 1995; Fazaeli *et al*., 2002). The bags were immediately tightened with nylon thread and kept in the fermentation house. Each bag contained about 12 kg wheat stubble or 10 kg date palm leaf.

A fermentation house 9×30×3 m was made in order to provide an optimal environment for fermentation and fungal growth. The temperature was maintained at 23±2°C using pad system and fan. The relative humidity was kept at 85±5% using an automatic humidity producer with sufficient aeration and light (Adamovic *et al*., 1998; Fazaeli *et al*., 2002). On the third day and after 2 weeks of incubation, bags were crushed on all sides to provide a sufficient aeration necessary for mycelial running and fruiting body formation. Two weeks after spawning (complete mycelial running stage) half of the bags were removed from the fermentation salon and exposed to sun drying. After the third (first stage of fruiting body formation) weeks of incubation, remaining bags were also exposed to sun drying.

The treatments were untreated wheat stubble (UWS), mycelium treated wheat stubble (MWS), fungal treated wheat stubble after harvesting of mushroom (FWS), untreated Date Palm Leaf (UDPL), mycelium treated date palm leaf (MDPL) and fungal treated date palm leaf (FDPL) after harvesting of mushroom.

In each stage, samples were taken from the treatments and dried at low heat oven (60°C), ground to pass through 1mm sieve size and stored in plastic bottle with lid. The samples were subjected to *in vitro* gas production method at 2, 4, 6, 8, 12, 24, 48, 72, 96 h of incubation period and total gas production (mL/0.5 g substrate) was estimated according to technique reported by Menke *et al*. (1979). Exponential model (Blümmel and Ørskov, 1993) was chosen to describe the kinetics of gas production from treatments. Rumen liquor was obtained from three ruminally fistulated bulls, maintained on wheat stubble based diet.

The data were subjected to factorial design using two way analysis of variance of SYSTAT software, release 11.0 (Copyright SYATAT Software, Inc., 2004). Differences between means were estimated by Pairwise Multiple Comparison Procedures, Duncan's Method (Cochran and Cox, 1992).

RESULTS

Chemical composition of treated and untreated wheat stubble and date palm leaves are shown in Table 1. Date palm leaves contained significantly higher CP, NDF and ADL and lower ADF than wheat stubble. Fungal treatment also affected chemical composition of the substrates.

The effect of fungal treatment of roughages on total, extent (b) and rate (c) of gas production and lag phase (lag) are presented in Table 2 and graphically illustrated in Fig. 1a and b. There were significant differences between treatments (control, mycelial and harvesting stages) in total gas production during the incubation period. Total gas production in control, mycelial and harvesting stages was 35.83, 29.50 and 27.45 mL, respectively. The volume of gas produced, irrespective of treatments, in wheat stubble (44.56 mL) was higher ($p<0.05$) than those measured in date palm leaves (17.29 mL). However, the reduction in gas production observed at mycelial and harvesting stages compared with those from control in wheat stubble was not observed in date palm leaves.

Table 1: Chemical composition of treated and untreated wheat stubble and date palm leaves

Source	OM (g kg^{-1})	CP (g kg^{-1})	NDF (g kg^{-1})	ADF (g kg^{-1})	ADL (g kg^{-1})	GE (kcal g^{-1})
Treatment						
Natural	908.38^{b}	38.20^{b}	630.25^{b}	465.25^{b}	84.75^{b}	4.27^{c}
Mycelium	919.75^{a}	41.10^{a}	670.50^{a}	518.50^{a}	97.12^{a}	4.41^{b}
Harvested	908.13^{b}	37.50^{b}	624.00^{b}	466.75^{b}	94.00^{a}	4.53^{a}
SEM	1.02	0.48	5.52	7.48	1.35	0.02
Substrate						
NW	921.33^{a}	36.22^{b}	667.83^{a}	487.83^{a}	60.67^{b}	4.34^{b}
DPL	902.83^{b}	41.61^{a}	615.33^{b}	479.17^{a}	123.25^{a}	4.47^{a}
SEM	0.83	0.39	4.51	6.11	1.10	0.02
Treatment and substrate						
NW×Natural	904.75^{d}	37.00^{c}	650.00^{a}	469.50^{c}	56.00^{e}	4.17^{c}
NW×Mycelium	936.75^{a}	38.50^{bc}	693.50^{a}	507.00^{b}	63.25^{d}	4.32^{b}
NW×Harvested	922.50^{b}	33.10$_{d}$	660.00^{a}	487.00^{b}	62.75^{d}	4.52^{a}
DPL×Natural	912.00^{c}	39.45^{b}	610.50^{a}	461.00^{c}	113.50^{c}	4.37^{b}
DPL×Mycelium	902.75^{e}	43.57^{a}	647.50^{a}	530.00^{a}	131.00^{a}	4.49^{a}
DPL×Harvested	893.75^{f}	41.80^{a}	588.00^{a}	446.50^{c}	125.25^{b}	4.54^{a}
SEM	1.44	0.68	7.81	10.58	1.91	0.03

^{a,b,c,d,e}values bearing different superscripts in a column differ significantly $p<0.05$. Natural = substrate without fungal treatment; NW = Wheat stopple, DPL = Date Palm Leaves

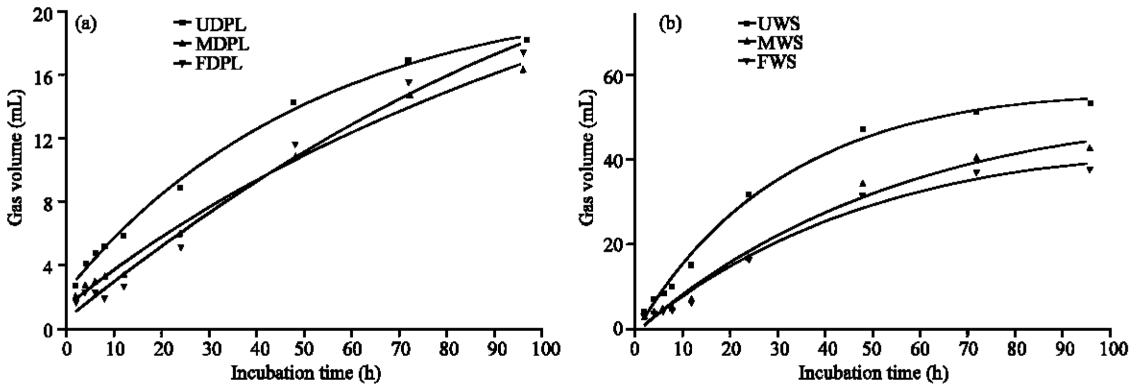

Fig. 1: *In vitro* cumulative gas production (mL/0.5 g) at different intervals of treated and untreated date palm leaves (a) and wheat stubble (b)

Table 2: *In vitro* total gas production (mL/0.05g) and kinetics of gas production of treated and untreated wheat stubble and date palm leaves

		Kinetic parameters		
Treatment	Total gas (mL)	b[1]	c[2]	Lag[3]
Natural	35.83^{a}	39.38	0.0261^{a}	-2.71^{a}
Mycelium	29.50^{b}	42.19	0.0138^{b}	-2.37^{a}
Harvested	27.45^{c}	40.91	0.0143^{b}	-0.31^{b}
SEM	0.49	1.84	0.0008	0.34
Substrate				
NW	44.56^{a}	52.15^{a}	0.0247^{a}	1.19^{a}
DPL	17.29^{b}	29.51^{b}	0.0114^{b}	-4.78^{b}
SEM	0.40	1.50	0.0006	0.28
Treatment×substrate				
NW×Natural	53.40^{a}	56.85^{a}	0.0338^{a}	0.88
NW×Mycelium	42.73^{b}	53.74^{a}	0.0190^{c}	1.28
NW×Harvested	37.55^{c}	45.85^{b}	0.0213^{b}	1.42
DPL×Natural	18.25	21.90^{d}	0.0185^{c}	-6.30^{a}
DPL×Mycelium	16.28	30.65^{c}	0.0085^{d}	-6.01^{a}
DPL×Harvested	17.35	35.98^{c}	0.0073^{d}	-2.04^{b}
SEM	0.69	2.60	0.001	0.48

^{a,b,c,d,e}values bearing different superscripts in a column differ significantly $p<0.05$. Natural = substrate without fungal treatment; NW = Wheat stopple, DPL = Date Palm Leaves. [1]b = Potential gas produced, [2]c = Fractional rate gas production (h^{-1}), [3]lag = Lag phase (h)

Table 3: Predicted effective degradability (%) of treated and untreated wheat stubble and date palm leaves

	Out flow rate (h^{-1})			
Treatment	0.02	0.04	0.06	0.08
Natural	23.34^{a}	16.78^{a}	13.14^{a}	10.85^{a}
Mycelium	17.41^{b}	11.20^{b}	8.28^{b}	6.58^{b}
Harvested	16.36^{c}	10.48^{c}	7.68^{b}	6.04^{b}
SEM	0.28	0.23	0.21	0.20
Substrate				
NW	27.68^{a}	18.73^{a}	14.01^{a}	11.11^{a}
DPL	10.39^{b}	6.91^{b}	5.38^{b}	4.53^{b}
SEM	0.22	0.19	0.17	0.16
Treatment×substrate				
NW×Natural	34.98^{a}	25.05^{a}	19.35^{a}	15.68^{a}
NW×Mycelium	25.20^{b}	16.13^{b}	11.70^{b}	9.08^{b}
NW×Harvested	22.88^{c}	15.00^{c}	10.98^{b}	8.58^{b}
DPL×Natural	11.70^{d}	8.50^{d}	6.93^{c}	6.03^{c}
DPL×Mycelium	9.63^{e}	6.28^{e}	4.85^{d}	4.08^{d}
DPL×Harvested	9.85^{e}	5.95^{e}	4.38^{d}	3.50^{d}
SEM	0.39	0.32	0.30	0.28

$^{a, b, c,d, e}$values bearing different superscripts in a column differ significantly $p<0.05$. Natural = substrate without fungal treatment; NW = Wheat stopple, DPL = Date Palm Leaves

Potential gas production (b) was not significantly affected by treatments, however, it was lower ($p<0.05$) in DPL (52.15 vs. 29.51). The "b" constant at harvesting stage compared to the control in WS was lower (45.85 vs. 56.85) however, this constant was significantly higher (35.98 vs. 21.90) in DPL at harvesting stage (Table 2).

Rate of gas production decreased ($p<0.05$) at harvesting stage compare to control in both WS and DPL. In addition to that "c" was higher ($p<0.05$) in WS than in DPL. Predictive Effective Degradability (ED) calculated at different outflow rates are shown in Table 3. Effective degradability at different outflow rates at mycelial and harvesting stages of WS and DPL were lower than their controls. However, ED of WS at different outflow rates were higher ($p<0.05$) than those obtained for DPL.

DISCUSSION

Total gas production was over 60% higher in WS than DPL, which indicated poor fermentability of DPL, evidently due to its higher lignin content (Singh *et al*., 2005). Additionally, increase in gas production during intervals from WS was higher than those from DPL. It may be a reasonable evidence of the availability of nutrients to microbes during fermentation period and more digestibility of WS in comparison to DPL. This was reflected in both b and c constants which were 43.41 and 53.85% higher in WS compared to DPL, respectively. Effective degradability of WS estimated at 2% outflow rate was 62.46% more than DPL. These results showed a significantly better digestibility of WS compared to DPL, possibly due to lesser accessibility of ruminal microbes to the fiber content of DPL.

Fungal treatment at both mycelial and harvesting stages resulted in 19.18 and 29.68% reduction in total gas production in WS. However, no significant change occurred in the total gas production due to fungal treatment in DPL. Such an interaction between roughages and fungal treatment observed in the present study has also been reported by others (Zadrazil andBrunnert, 1980; Jung *et al*., 1992; Safari *et al*., 2005; Okano *et al*., 2006). Our results of *in vitro* gas production were not in agreement with Miki andOkano (2005), who reported that fungal treatment of wheat and rice straw increased *in vitro* gas production by 20 and 11%, respectively. Okano *et al.* (2006) also reported significant increase in *in vitro* gas production of bagasse cultured with *Lentinula edodes* and two strains of *Ceriporiopsis subvermispora* whereas such alteration was not recorded by *Pleurotus eryngii, Pleurotus salmoneostramineus*. There is very little report on gas production of either WS or DPL treated with fungi. However, high correlation between the *in vitro* gas production and DM or OM digestibility has been found (Miki and Okano, 2005; Hozhabri and Singhal, 2006). Variable results have been reported on the effect of white rot fungi on DM digestibility of different low quality roughages depending on the fungi species and substrate (Zadrazil and Brunnert, 1980; Hatakka, 1983; Zadrazil, 1985; Muller and Trosch, 1986; Jung *et al*., 1992). However, many of reports have demonstrated significant increase in *in vitro* DM digestibility of roughages (Rouzbehan *et al*., 2001; Fazaeli *et al*., 2004: wheat straw; Miki and Okano, 2005: rice and wheat straw; Soilman *et al*., 2003; Hamza *et al*., 2003: corn stalks).

CONCLUSION

As a result of this experiment, it can be concluded that *Pleurotus florida* treatments of both WS and DPL resulted in no improvement in gas production properties of these two roughages.

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