Effect of solid-state fermentation by oyster mushroom (*Pleurotus florida*) on nutritive value of some agro by-products

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ABSTRACT
The present research aims to investigate the effect of *Pleurotus florida* fungi cultivation on chemical composition, gas production characteristics, organic matter digestibility (OMD), metabolizable energy (ME), net energy lactation (NEL) and short-chain fatty acid (SCFA) of some agro by-products (rice straw, wheat straw, barley straw, soybean straw, canola straw, pea straw and rice husk). Dry matter, organic matter and neutral detergent fibre were significantly decreased upon with fungi application in all treatments ($p < 0.01$). Fungal treatment did not cause significant difference in the amount of acid detergent fibre (ADF) in soybean straw, canola straw, pea straw and rice husk ($p > 0.05$), but significantly decreased ADF in rice straw, wheat straw and barley straw ($p < 0.05$). In experimental treatments, fungi cultivation did not vary significantly in acid detergent lignin and ether extract content ($p > 0.05$). Processing significantly increased ash and crude protein in all treatments ($p < 0.01$). The amount of gas production was not significantly affected by fungi cultivation in wheat straw, soybean straw, canola straw and rice husk; however, it was significantly increased upon processing in rice straw, barley straw and pea straw ($p < 0.05$). The amount of OMD in rice husk was not changed significantly, but processing significantly increased OMD in other treatments ($p < 0.05$). Fungal cultivation significantly improved ME in all treatments ($p < 0.05$). The amounts of NEL and SCFA were significantly increased with treatment by fungi (except rice husk) in all substrate ($p < 0.01$). The results of this study generally indicated that nutritive value of agro by-products was improved upon fungal treatment.

1. Introduction
The high cost of animal feed in semi-arid and arid countries is a major problem that limits livestock production. Therefore, using agro by-products as alternative feed resources is a reasonable strategy in low-input systems (Dayani et al. 2012). Crop residues are a large potential energy source for ruminants, but they are characterized with very poor intrinsich feeding value, because of low digestible dry matter and protein content. The rumen microorganisms are able to exude enzymes that have potential to directly hydrolyse cellulose and hemicel lulose in the rumen (Yalchi & Hajieghrabi 2011), but the presence of lignin in the complex network that is formed by cellulose and hemicel luloses reduces their digestibility because of lacking lignin-degrading activity in the rumen (Zadrazil 1985; Otj en et al. 1987; Falcon et al. 1995). Therefore, crop residues’ lignocellulosic bond degradability is not very efficient in rumen. Hence, several physical and chemical processing techniques have been studied for improving its nutritive value (Matsuzaki et al. 1994; Rahal et al. 1997). In spite of advantages of these methods, they are not cost-effective, environmentally friendly and also require application of state-of-the-art technology (Leng 1991). These factors restrict their application, particularly at small farm scale. In recent times, biological delignification of agricultural residues by solid state fermentation (SSF) has been considered, thanks to its capacity to remove lignin preferentially (Moyson & Verachtert 1991). Fungal treatment could be an approach to improve crop residues for ruminant nutrition (Arora et al. 1994; Zadrazil et al. 1996). During SSF by fungi, OM and detergent fibre content of substrate could be reduced and the lignin selectively is removed from the lignocellulosic complex (Singh et al. 1990; Kundu 1994). However, such changes depend on the strain of fungi and the cultural conditions (Tripathi & Yadav 1992). Some attempts have been made to identify species of white-rot fungi that can grow on these by-products, which in turn improves their nutritive value (Yamakawa et al. 1992). Among the edible white-rot fungi, the *Pleurotus* (P) species has been identified as an efficient species for this purpose (Zadrazil et al. 1996). The potential of some species of Pleurotus fungi such as *Pleurotus ostreatus* and *Pleurotus eryngii* to reduce indigestible cell-wall components and increase DMD of straw has been reported (Agosin et al. 1986; Singh et al. 1990). Among different in

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vitro feed analyses, the gas production technique is of specific importance. The amount of gas produced is closely related to the digestibility and the energetic value of diets for ruminants (Shrivastava et al. 2014). Despite much research about the effect of SSF on nutritive value of agro by-products, there is little information about the effect of some species. Therefore, this study was conducted to investigate the effect of Pleurotus florida fungi cultivation on chemical composition, gas production characteristics, OMD, metabolizable energy (ME), net energy lactation (NEL) and short-chain fatty acid (SCFA) of some agro by-products (rice straw, wheat straw, barley straw, soybean straw, canola straw, pea straw and rice husk) using in vitro gas production technique.

2. Materials and methods

2.1. Treatment of agro by-products and chemical analysis

Agro by-products were collected from local farms in Gorgan, Golestan province, Iran. Pleurotus florida fungi were used for treating those residues. Treatment was carried out in 1000 ml bottles. The 50 g each sample was placed in individual bottle and water added to give moisture content of about 85%. The bottles were autoclaved at 121°C for 20 min. Each bottle was inoculated with 5% (w/v) spawns of Pleurotus florida fungi (Jahromi et al. 2010). Each treatment had four replicates. The bottles were incubated in an incubator in which temperature was automatically adjusted to 25°C and relative humidity was kept at 78 ± 5%. After 21 days, samples were dried in oven (60°C) in order to stop fungi growth and then chemical composition was determined. DM was determined by drying the samples at 105°C overnight and ash was determined by igniting the samples in muffle furnace at 550°C for 8 h. OM was calculated by subtracting Ash from DM. EE was determined by Soxhlet extraction method (AOAC 2002). Neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) contents were measured by Fiber-Tec system (Van soest et al. 1991). Nitrogen (N) content was measured by Kjeldahl method and crude protein (CP) was calculated as

\[ \text{CP} = \frac{\text{N} \times 6.25}{\text{DM}} \]  (AOAC 2002)

Soest (1970), without tryppticase was immediately added in 1:4 (v/v) ratio. The solution contained micro-minerals (CaCl2·H2O, MnCl2·4H2O, CoCl2·6 H2O, FeCl3·6 H2O), macro-minerals (Na2HP04 anhydrous, KH2PO4 anhydrous, MgSO4·7 H2O) and reducing solution (1N NaOH, Na2S·9H2O), in addition to resazurin and buffer reagent of NH4HCO3 and NaHCO3 (Patra & Yu 2013; Salem et al. 2014). These serum bottles were incubated at 39°C for 96 h in an incubator. Volume of gas produced was recorded at incubation times of 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 h after inoculation as per reading pressure technique (Salem et al. 2014).

2.3. Measurement

Kinetic parameters of gas production were estimated by fitting GP results (ml/g DM) according to the model described by Ørskov and McDonald (1979)

\[ P = a + b (1 - e^{-ct}) \]

where \( P \) is the volume of GP at time \( t \) (ml/g DM), \( a \) is volume of GP from soluble fraction (ml/g DM), \( b \) represents the volume of GP from insoluble but fermentable fraction (ml/g DM), \( c \) is the rate of GP (/h) from the fraction \( b \), \( t \) is the time (h) and \( e \) = 2.7182.

ME (MJ/kg DM) and in vitro organic matter digestibility (OMD, %) were estimated according to the formula given in Menke et al. (1979):

\[ \text{ME (MJ/kg DM)} = 2.20 + 0.136 \times \text{GP} + 0.057 \times \text{CP}, \]

OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA,

where DM and CP are expressed in (crude protein) in percent; XA, ash in percent; and GP, the net gas production in millilitres from 400 mg dry sample after 24 h of incubation.

NEL was calculated (Menke & Steingass 1988) as

\[ \text{NEL (MJ/kg DM)} = 0.096 \times \text{GP} + 0.0038 \times \text{CP} + 0.000173 \times \text{EE}^2 + 0.54, \]

where GP is net gas production in millilitres from 400 mg dry sample after 24 h of incubation, CP and EE in percent.

SCFA was calculated (Getachew et al. 2002) as

\[ \text{SCFA (m mol/mg DM)} = 0.0222 \times \text{GP} - 0.00425, \]

where GP is the net gas production in millilitres from 400 mg dry sample after 24 h of incubation.

2.4. Statistical analyses

For all data, a completely randomized design with a 2 × 7 factorial arrangement was used. The experimental factors included fungi application (Control and Pleurotus florida fungi) and crop residues (rice straw, wheat straw, barley straw, soybean straw, canola straw, pea straw and rice husk). Each parameter was measured with four replicates for chemical composition. Three replicates were considered in gas production. The data obtained were analysed for parametric statistics, including analysis of variance using the general linear model procedure.
and the differences among treatments means were compared by Tukey’s test. Statistical analyses were performed using SAS (2002) software package.

3. Results

3.1. Chemical composition

The chemical composition of experimental treatments is shown in Table 1. In all treatments the amount of dry matter and organic matter were significantly decreased upon SSF application (p < 0.01). SSF significantly increased ash and CP content of all treated crop residues (p < 0.01). However, EE in crop residues did not affected by SSF. SSF lowered DM and OM content of pea straw more than other substrates. On the other hand, ash content was increased under treatment more than other crop residues used in this experiment. Among experimental treatments, the highest increase in the protein content under SSF occurred in barley straw.

3.2. Cel-wall components

The cel-wall components of experimental treatments are presented in Table 2. The amount of NDF significantly decreased upon fungal treatment in all crop residues (p < 0.01). The most reduction in NDF content among experimental feedstuffs was related to rice husk. ADF in soybean straw, canola straw, pea straw and rice husk (p > 0.05) did not get affected by fungal treatment in a significant manner but significantly decreased ADF was noted in rice straw, wheat straw and barley straw (p < 0.05). SSF reduced ADF content of barley straw more than that of other crop residues. Fungi cultivation did not led to significant difference in ADL content of substrates.

3.3. In vitro gas production

3.3.1. Gas volume

The in vitro gas production over the period of 96 h is shown in Table 3. Fungal treatment increased the amount of gas produced from all crop residues after 96 h incubation (p < 0.01). SSF increased the amount of gas produced from barely straw more than other substrates.

3.3.2. Gas production characteristics

The effect of fungal treatment on gas production characteristics of crop residues is shown in Table 4. Treatment by fungi significantly increased fraction of gas production in rice husk, but this fraction did not get affected by treatment in other crop residues. Fraction of gas production in barley straw was significantly (p < 0.05) increased by fungal treatment, but this fraction did not significantly changed in other agro by-products after fermentation. Potential extent of gas production (a + b) in wheat straw, soybean straw, canola straw and rice husk was not affected by fungi treatment, but significantly enhanced it in rice straw, barley straw and pea straw (p < 0.05). SSF improved (a + b) content of gas production in barley straw more than other crop residues (Table 4).

3.3.3. In vitro OMD, ME, NEL and SCFA

Table 5 shows the effect of SSF on OMD, ME, NEL and SCFA of crop residues. Fungal treatment did not affect rice husk OMD, but significantly improved it in other substrates (p < 0.05). This treatment increased pea straw OMD more than that in other crop residues. Canola straw ME content did not get significantly affected by SSF than that of other substrates (p < 0.05). SSF increased ME in soybean straw more than in other crop residues. NEL content of rice husk was not affected by the treatment, but SSF increased the same content substantially in other substrates (p < 0.01). Rice husk SCFA did not change upon fungal treatment, but the same did significantly increase in other crop residues (p < 0.01). SSF increased SCFA content in pea straw more than in other substrates.
**Table 3. The volume of GP (ml/g DM) from experimental treatments in different incubation times.**

| Incubation times | 2 | 4 | 6 | 12 | 24 | 48 | 72 | 96 |
|-----------------|---|---|---|----|----|----|----|----|
| Rice straw      | U | 1.43 | 2.49 | 4.50 | 6.48 | 11.17 | 17.83 | 30.00 | 36.67 | 39.17 |
|                 | T | 2.87 | 6.00 | 8.17 | 10.00 | 13.33 | 22.17 | 30.89 | 38.22 | 45.66 |
| Wheat straw     | U | 1.37 | 4.00 | 6.00 | 8.50 | 12.67 | 22.67 | 35.67 | 39.00 | 44.00 |
|                 | T | 3.54 | 6.83 | 9.34 | 11.83 | 17.00 | 31.00 | 40.34 | 42.34 | 48.02 |
| Barley straw    | U | 3.04 | 6.50 | 9.17 | 12.00 | 18.00 | 31.83 | 42.17 | 45.00 | 46.67 |
|                 | T | 3.57 | 6.50 | 10.00 | 14.50 | 22.67 | 35.83 | 42.19 | 47.21 | 55.00 |
| Soybean straw   | U | 3.37 | 7.00 | 9.00 | 11.17 | 17.67 | 32.00 | 42.01 | 46.89 | 49.56 |
|                 | T | 8.47 | 11.33 | 16.50 | 19.30 | 24.00 | 30.00 | 40.67 | 47.78 | 49.25 |
| Canola straw    | U | 1.70 | 4.33 | 5.50 | 7.14 | 9.84 | 13.33 | 16.00 | 22.18 | 29.65 |
|                 | T | 1.87 | 6.17 | 9.84 | 13.33 | 16.00 | 22.18 | 39.65 | 45.11 | 48.88 |
| Pea straw       | U | 4.04 | 7.83 | 10.34 | 12.6 | 21.50 | 34.50 | 43.85 | 47.50 | 51.84 |
|                 | T | 8.37 | 16.83 | 27.67 | 32.50 | 37.17 | 45.00 | 53.34 | 54.50 | 56.69 |
| Rice straw      | U | 0.87 | 3.00 | 4.17 | 5.33 | 6.78 | 6.50 | 7.17 | 7.50 | 7.50 |
|                 | T | 1.37 | 3.33 | 4.67 | 5.50 | 6.00 | 6.50 | 7.84 | 8.64 | 9.00 |
| p value         | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| SEM             | 0.22 | 0.26 | 0.36 | 0.46 | 0.69 | 0.67 | 0.75 | 0.84 |

Note: Means in the same column with the different superscript are significantly different (p < 0.01).

**Table 4. Effect of treatment by fungi on kinetic parameters of gas production of crop residues.**

| Items           | a     | b     | a + b  | c     |
|-----------------|-------|-------|--------|-------|
| Rice straw      | -1.23 | 45.40 | 44.17  | 0.02  |
| Wheat straw     | -1.51 | 46.50 | 45.38  | 0.04  |
| Barley straw    | -0.69 | 48.03 | 47.34  | 0.04  |
| Soybean straw   | -1.25 | 51.68 | 50.42  | 0.04  |
| Canola straw    | -0.17 | 51.82 | 51.64  | 0.05  |
| Pea straw       | -1.66 | 49.16 | 47.49  | 0.04  |
| Rice husk       | -1.02 | 52.11 | 51.08  | 0.04  |
| SEM             | 0.2   | 0.08  | 0.18   | 0.0001|

Note: Means in the same column with the different superscript are significantly different (p < 0.01) and (p < 0.05).

4. Discussion

4.1. Chemical composition

Reduction in DM and OM content of fungal-treated crop residues has been reported by Fazaeli et al. (2006), Akinfemi et al. (2010) and Rahman et al. (2011). Loss of DM and OM in agro-by-products after treatment by fungi could be correlated with consumption of substrate carbohydrates in cell wall. Also, this loss in DM and OM content is related to the extent of fungal growth (Mukherjee & Nandi 2004). Therefore, higher reduction of DM and OM content in pea straw than in other substrates can be related to its higher available carbohydrates and the extent of fungal growth on it. An increase in CP content in wheat straw treated with *Pleurotus* species has also been reported (Ardon et al. 1996; Zadrazil et al. 1996; Fazaeli et al. 2004). The increase observed in the CP content of the fungal-treated crop residues probably can be attributed to the addition of fungal protein during fermentation. Presumably, CP increase is stemmed from hydrolysis of carbohydrates and its subsequent use as a carbon source to synthesize fungal biomass that rich in protein (Akinfemi et al. 2010). Also, CP increase by SSF may be due to the capturing excess nitrogen by fermentation (Sallam et al. 2007), foregoing results suggest that the fermented substrates are good source of protein for livestock.

4.2. Cel-wall component

Reduction in the fibre fraction of fungal-treated substrate has been reported frequently in literature by Nasehi et al. (2014), Lynch et al. (2014) and Shrivastava et al. (2014). The decrease in the amount of NDF and ADF of fungal-treated crop residues denotes on the degradation of the cel-wall component of the substrates produced by extra cellular enzymes of fungus (Akinfemi et al. 2010). Fungi have two kind of extra cellular enzymes. The volume of GP (ml/g DM) from different treatments in different incubation times.

**Table 5. Effect of treatment by fungi on OMD (%), ME (MJ/kg DM), NEL (MJ/kg DM) and SCFA (m mol / mg DM) of crop residues.**

| Items           | OMD   | ME     | NEL    | SCFA  |
|-----------------|-------|--------|--------|-------|
| Rice straw      | 40.10 | 4.82   | 2.37   | 0.39  |
| Wheat straw     | 41.76 | 5.25   | 2.87   | 0.50  |
| Barley straw    | 49.99 | 6.77   | 3.75   | 0.70  |
| Soybean straw   | 49.34 | 6.83   | 3.80   | 0.70  |
| Canola straw    | 49.38 | 6.25   | 3.39   | 0.60  |
| Pea straw       | 49.99 | 7.29   | 4.11   | 0.76  |
| Rice husk       | 32.29 | 4.36   | 1.94   | 0.14  |
| SEM             | 0.01  | 0.01   | 0.0001 | 0.0001|

Note: Means in the same column with the different superscript are significantly different (p < 0.01) and (p < 0.05).

U: untreated; T: treated by fungi; OMD: organic matter digestibility; OMD (%) = 14.88 + 0.889 GP + 0.45 CP + 0.0651 XA; ME: metabolizable energy; ME (MJ/kg DM) = 2.20 + 0.136 GP + 0.057 CP; NEL: net energy lactation; NEL (MJ/kg DM) = 0.096GP + 0.038CP + 0.000173EE2 + 0.54; SCFA: short-chain fatty acid; SCFA (m mol / mg DM) = 0.022GP - 0.00425. p value = probability value, SEM = standard error of means.
enzymatic systems: the hydrolytic system which produces hydrolyses that are responsible for polysaccharide degradation and a unique oxidative and extracellular ligninolytic system, which degrades lignin and opens phenyl rings (Sanchez 2009).

4.2. In vitro gas production

4.2.1. Gas volume

After 96 h incubation upon fungal treatment, gas produced from all crop residues was increased \( p < 0.01 \) (Table 3). These results supported by Akinfemi et al. (2010) report about bioconversion of maize husk by white-rot fungi. There are many factors affecting the amount of gas produced during fermentation, such as the nature and level of fibre and potency of the rumen liquor used for incubation (Babayemi 2007). Generally, gas production reflects degradable carbohydrate and, therefore, the amount of gas produced depends on carbohydrates nature (Demeyer & Van Nevel 1975; Bummel & Becker 1997). NDF and ADF have a negative correlation with gas production at all incubation times and estimated parameters. The reduction in the value of NDF and ADF tends to increase in the microbial activity through increasing favourable environmental conditions as incubation time progresses. Therefore, the lower levels of fibre fraction in the treated substrates increased amount of gas production (Akinfemi et al. 2010).

4.2.2. Gas production characteristics

Increased \( b \) fraction of gas production after treatment by fungi has been reported by some literature, i.e. Rodrigues et al. (2008), Okano et al. (2009) and Akinfemi et al. (2010). Such increase in barley straw presumably is a reflection of reduction in fibre fraction. In addition, this result may be related to improved carbohydrates fraction availability for microbial fermentation (Akinfemi et al. 2010). Increase in \( (a+b) \) content of gas production were supported by other researchers (Rodrigues et al. 2008; Okano et al. 2009; Rahman et al. 2011). Improved \( (a+b) \) content of gas production implies that the fermented crop residues were highly available in the rumen. Fibrous constituents affected in vitro gas production negatively; therefore increased \( (a+b) \) content of gas production upon SSF can be related to reduction in cel-wall components (Akinfemi et al. 2010).

4.2.3. In vitro OMD, ME, NEL and SCFA

Rice husk OMD was not changed significantly upon fungal treatment, but significantly improved in other substrates \( p < 0.05 \) (Table 5). These findings are supported by Mukherjee and Nandi (2004), Okano et al. (2009), Sharma and Arora (2010) and Brozzoli et al. (2010) reports. Improved OMD by fungal treatment is probably associated to reduction in cel-wall components and an increase in CP content of substrate after fermentation (Mukherjee & Nandi 2004, Akinfemi et al. 2010, Sharma & Arora 2010). Canola straw ME content was not affected by SSF in a significant manner, but it is found to significantly increase in other substrates \( p < 0.05 \) (Table 5). NEL content of rice husk did not vary upon treatment, but SSF significantly improved the same in other substrates \( p < 0.01 \) (Table 5). A number of factors could be responsible for ME and NEL improvement in crop residues treated with fungi, such as high gas production in the treated substrate, reduction in cel-wall components and an increase in CP content of treated substrate (Sallam et al. 2007; Akinfemi et al. 2010). Fungal treatment did not led to significant variations in rice husk SCFA, but significantly increased it in other crop residues \( p < 0.01 \) (Table 5). SCFA improvement is related to increasing 24 h gas volume by fungal treatment. Increasing 24 h gas volume show that treated crop residues are more available for rumen microorganisms. This can be a reason for SCFA increasing in fermented crop residues (Akinfemi et al. 2010).

5. Conclusion

Solid-state fermentation by *Pleurotus florida* fungi resulted in a reduction of the cel-wall components and increased CP content in experimental crop residues. Upon treatment OMD, ME, NEL and SCFA were improved. In terms of foregoing results, it can be suggested that the treatment of crop residues by application of fungi will be promising on conversion of agro by-products to higher quality ruminant feed, enhancing their digestibility by ruminants.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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