

In vitro Determination of Nutritional Value of Compost and Stem of the White Button Mushroom

Research Article

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ABSTRACT

The aim of this study was to determine the chemical composition of stem and compost of the button mushroom to estimate metabolizable energy (ME) and net energy of lactation (NE₁) using gas production method and to determine the amount of dry matter disappearance (DMD) using *in vitro* technique. This study was conducted in a completely randomized design with three treatments of mushroom stem, alfalfa, and compost. The data were analyzed by SAS software. Dry matter (DM), crude protein (CP), crude ash (CA), ether extract (EE), acid detergent fiber (ADF) and neutral detergent fiber (NDF) content of the stem, compost and alfalfa were: 10.3, 16.8, 8.6, 3.48, 31.5, and 13.4; 42.4, 12.8, 42.41, 3.14, 21.5, and 16.2; 96.4, 12.6, 9, 2.2, 38.6, and 32.3, respectively. The highest and the lowest amounts of gas production after 120 h of incubation were obtained for mushroom stem and compost, respectively. Gas production potential (a+b) of mushroom stem, alfalfa, and compost were obtained as 223.01, 200.0, and 114.53 mL gas/g DM, respectively. Gas production rates for mushroom stem, compost, and alfalfa were 0.07, 0.03, and 0.08/h, respectively. The highest values of ME and NE₁ were received for mushroom stem (8.01 and 4.82 MJ/kg DM, respectively). DMD was higher and lower in the mushroom stem and compost, respectively. The results demonstrated that mushroom stem had a higher nutritive value compared with the other experiment feeds and can be used in ruminant ration formulation.

KEY WORDS chemical composition, compost, degradability, gas production, mushroom stem.

INTRODUCTION

One of the goals of cultivating mushrooms is to provide human edible protein and among various edible mushrooms, button mushroom comprises 38% of total mushroom cultivation worldwide (Farsi and Gordan, 2010). Two by-products are obtained from button mushroom farms, one of which is mushroom stem being separated from the main product and discarded during the mushroom crop harvest due to lack of marketability in fresh mushroom crop products. This by-product contains a compound almost similar to that of the mushroom and can be used for ruminant feeding. The second by-product is button mushroom cultivation bed called compost, which is discarded after harvesting. According to a previous study, changes during compost processing and growth of button mushrooms increased dry matter disappearance (DMD) and rice straw protein in the rumen (Kim *et al.* 2011). Ehtesham and Vakili (2015) replaced wheat straw with button mushroom compost at levels of 15, 25, and 35% in Kurdish lamb ration and reported that this replacement had no negative effects on blood metabolites; they recommended a 25 percent compost replacement instead of wheat straw in the diet. Barati (2014) replaced alfalfa with the silage of white button mushroom compost at 7.2% and 15.9% in the diet of Mehraban lambs, which had no significant negative effects on average daily

weight gain, feed conversion efficiency, and average lamb final weight during a 60 d period. They concluded that the silage of button mushroom compost could be a good substitute for a part of dietary forage portion. Addition of mushroom stems (10% and 20%) to the diet of Mehraban male lambs had no negative effects on lamb growth performance, digestibility of dry matter (DM) and other nutrients in the ration. It was found that mushroom stems with high crude protein (CP) content and appropriate amount of energy could replace dietary protein sources (Yousefi, 2016). Therefore, regarding to nutritive value and low costs of these agricultural by-products, it can be used in diets of ruminants.

Identification and evaluation of feeds as well as determination of animal nutrition requirements are two important factors in a high-yield and economic production. For this reason, digestive experiments are of particular importance in determining the nutritional value of feeds in animal nutrition. *In vitro* methods are widely used for prediction of feed digestibility and are a tool for assessing the quality of feeds.

A high correlation was also reported between nylon bags and *in vitro* methods in determining the amounts of dry matter and protein disappearance (Paya *et al.* 2008). Considering the high correlation between two methods mentioned above, *in vitro* method can be introduced for estimation of the amount of feed DMD.

Regarding to absence of more information about of mushroom wastes, this study was designed and conducted to obtain information about nutritive value, digestion kinetics, gas production parameters and DMD of white button mushroom compost and stems in ruminant nutrition.

MATERIALS AND METHODS

The compost was prepared at the end of mushroom harvest period in a field in Ghezeljeh Maidan village, Bostanabad (Eastern Azerbaijan province). The compost samples were selected from different levels of the shelves at different areas of the cultivation room and dried at room temperature to prepare air-dried samples, after which each sample was milled with an appropriate sieve. To prepare the samples of edible white mushroom stem from the same field, edible mushroom stem wastes was prepared after daily mushroom harvesting. The mushroom stem was separated manually from the bed soil and placed at room temperature to prepare air-dried samples without washing. Dry alfalfa was used to compare the nutritive value and chemical composition of these two products with other conventional feeds. Alfalfa was obtained from a dairy farm forage store located in the central part of Tabriz. Approximate analysis of the samples, including DM, CP, ether extract (EE) and crude ash (CA) was performed according to the methods recommended by AOAC (2005). The values of acid detergent fiber (ADF) and neutral detergent fiber (NDF) were determined according to Van Soest *et al.* (1991).

Gas production was measured by the method of Fedorak and Herodi (1983). In this method, fluid displacement in calibrated test tubes, measured by the gas pressure produced in glasses containing rumen fluid and feed samples, represents the amount of produced gas. Rumen fluid was obtained from two of Gezel fistulated, adult one-year-old male rams (50±1 kg), kept separately in individual metabolic cages. To perform this test, 300 mg of each feed, previously milled with a 2 mm diameter pore mill, weighed carefully and poured into 50 mL sterile glass vials. Three replicates were considered for each sample. Two hours after a morning meal, rumen fluid was obtained from two fistulated sheep fed with a concentrated feed and alfalfa for two weeks. The fluid was transferred to the laboratory after being filtered through a four-layer grid inside a flask containing CO₂ at 39 °C. A mixture (20 mL) of rumen liquid and McDougall (1948) buffer at 1:2 ratio (one portion of rumen fluid and two portions of buffer) was taken from an Erlen already placed on a heater at 39 °C under constant CO2 gas flow, and was poured into the vials previously reached the desired temperature to prevent heat shock. The glass vials containing the experiment treatment were anaerobicized by CO₂ and riveted tightly with plastic caps and aluminum silica to be impermeable by the air. To correct the gas produced from rumen fluid, five glass jars were considered with no feeds containing only rumen fluid and the buffer. All prepared vials were transferred to a shaker incubator at 120 rpm and 39 °C. The amount of produced gas was recorded at 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h after incubation (Gallo et al. 2016). The gas production components were determined by the equation:

 $P = A (1 - e^{-ct})$

Where:

- P: gas production at time t.
- A: gas production of soluble and insoluble fractions.
- c: gas production rate.
- t: fermentation time..

In vitro disappearance method was used to determine DMD, which was similar to the gas production method, except that a hypodermic needle was placed on the rubber cap of the glass vials for the gas exit. Rumen fluid was collected from bovine rumen immediately after slaughter at Tabriz industrial slaughterhouse and then transferred to the laboratory. Three replicates were assigned to each treatment. For each time series, three blank samples were also considered to deduct the amount of rumen fluid during the calculations (Paya *et al.* 2008). To determine the DM, the samples were removed from the incubator after 4, 8, 12, 24 and 48 h and immediately transferred to a freezer to inhibit the activity of microorganisms. During the experiment and after freezing, the vials were centrifuged at 2500 rpm for 10 minutes, the floating portion/supernatant was separated and the residue was washed with a buffer (sodium hydrogen phosphate, potassium dihydrogen phosphate, NaCl, and distilled water). The vials were re-centrifuged at 2500 rpm for 10 min, the supernatant was separated, and the residue was transferred to an oven. The amounts of DM and DMD were measured after drying samples at 105 °C (Paya *et al.* 2008).

DM degradability coefficients of the samples were determined using the exponential equation:

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

Where:

P: level of degradation at time t.

a: readily soluble fraction.

b: insoluble fraction but degradable in rumen.

c: rate of degradation of b per hour.

t: time of incubation.

Effective degradability of the samples was calculated using Orskov and MacDonald (1979) equation ED= $a + {(b \times c) / (c+k)}$, taking into account the output rates of 0.02, 0.05, and 0.08 per hour. In this equation, ED is effective degradability and k is the constant of digested leachate outflow rate from the rumen. Other abbreviations are similar to those described in the preceding equation.

ME, NE_l, and organic matter digestibility (OMD) contents of the samples were calculated using the equations presented by Menke *et al.* (1979) and Menke and Steingass (1988). The amounts of short-chain fatty acids (SCFA) were calculated based on Getachew *et al.* (2002).

Finally, the data obtained from this experiment were analyzed by SAS (2003) software with ANOVA procedure in a completely randomized design with three treatments of alfalfa, stem, and compost of button mushrooms in five and three replications for evaluating gas production and disappearance of DM, respectively. The statistical model used here was:

$$Y_{ij} = \mu + T_i + e_{ij}$$

Where:

 Y_{ij} : volume of produced gas (mL/g DM). μ : total mean. T_i : treatment i effect. e_{ij} : test error.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of the experiment feeds is presented in Table 1. In previous studies, mushroom stem CP levels were reported about 19% (Nasiri *et al.* 2013) and 24.5% (Marino *et al.* 2010), which is different from current study.

Marino *et al.* (2010) reported a CP of 24.5% in the mushroom stem, which is in the contrast with that we recorded in this present, due to variance in used coefficient for calculation of CP (8.48 *vs.* 6.25). However, a coefficient of 4.38 was used in other studies (Nasiri *et al.* 2013) to calculate the amount of crude protein.

In the present study, a crude ash content of 8.6% was estimated for the mushroom stem. Some studies reported different values for crude ash such as 10.2% (Marino *et al.* 2010), 9.5% (Nasiri *et al.* 2013), and 7.7% (Yousefi, 2016). The existence of soil in this residue and different sampling methods may be caused different values in above mentioned studies. In our research, the ether extract level (3.48%) of the mushroom stem was in contrast to those of other studies, including 3.7% (Marino *et al.* 2010) and 2% (Nasiri *et al.* 2013), which can be due to differences in the test mushroom species.

The content of NDF in the mushroom stem was reported from 34.4% (Marino et al. 2010) and 34.9% (Yousefi, 2016). This difference may be due to variance in the mushroom growth stage, experimental mushroom cultivar, and the storage conditions in the mushroom growing room. The contents of mushroom cap and stem are different depending on the growing room conditions such as temperature, air flow rate or slightly delayed harvesting conditions (high growth rate, bulky or thin stems). Any report for NDF of this mushroom stem was not found in the literature reviews. Mushroom compost DM contents were reported from 35.1% (Fazaeli and Shafyee, 2005) and 83% (Ehtesham and Vakili, 2015). Their different DM levels with the present study may be due to variety in type of drying process such as feeding of air-dried compost DM fed to animals, which is not related to compost at the time of evacuation from the mushroom growing room containing high moisture content as one of the important principles of white mushroom cultivation.

	Chemical composition						
Feed	DM	СР	CA	EE	NDF	ADF	
Stem of mushroom	10.3	16.8	8.6	3.4	21.5	16.2	
Compost	42.4	12.8	42.4	3.1	31.5	13.4	
Alfalfa	96.4	12.6	9	2.2	38.6	32.3	

Table 1 Chemical composition (% DM) of the experiment feeds (n=3)

DM: dry matter; CP: crude protein; CA: crude ash; EE: ether extract; NDF: neutral detergent fiber and ADF: acid detergent fiber.

Our estimated compost CP (12.8%) is in consistent with that reported by Ehtesham and Vakili (2015), but it is different from those of reported by Fazaeli and Shafyee (2005) and Fazaeli and Talebian (2006), which can be attributed to differences in the sampling methods, mushroom growth rate, number of harvest periods, and differences in the initial compost chemical composition.

The content compost ash was achieved about 42.4% in the present study; while, this value was obtained 37%, 35.1% and 35.1% in the studies of Fazaeli and Shafyee (2005), Fazaeli and Talibian (2006), and Ehtesham and Vakili (2015), respectively.

The researchers attributed the high compost ash content to the consumption and discharge of straw organic matter by the mushroom (Fazaeli and Talibian, 2006; Ehtesham and Vakili, 2015). They argued that straw is associated with the soil in the white mushroom cultivation system, and the compost top layer is covered with soil for mushroom cultivation. Despite the separation of this layer prior to its use in livestock feeding, they found it impossible to be completely separable.

It should be noted that the difference in ash content can be one of the factors affecting different results of other nutrient compounds of compost between the present study and other studies. High ash content in a feed have an adverse relationship with energy content, it would also affect other nutrients being expressed as a percentage of DM.

The amount of EE in current study was 3.14%, whereas, a level of 1.26% was reported by Fazaeli and Shafyee (2005). The difference in EE values may be due to differences in the initial composition of the mushroom compost, also the amount of white mushroom residues associated the mycelium growth rate in the grown cultivars.

Compost NDF and ADF levels in our study were different from those reported by Ehtesham and Vakili (2015) (28.2% and 9.7%) and Fazaeli and Talebian (2006) (27.8% and 21%), respectively.

The discrepancy between the above levels can be explained by the fact that compost is the main source of mycelium nutrition resulting mushroom production. Moreover, many factors are involved in the mushroom growth at different stages resulting changes in the amount of nutrients in compost.

In vitro gas production

The gas production parameters of experiment feeds are given in Table 2. Among the experiment treatments at the end of 120 h of incubation, the higher and lower amount of gas was produced in the mushroom stem and compost, respectively (P<0.05). This is in contrast to that reported by Yousefi (2016) as higher gas production (281 mL/g DM) was achieved in alfalfa, whereas lower value was obtained in mushroom stem (263 mL/g DM) at the end of a 144 h incubation. The amount of mushroom stem gas production was 228 mL at 120 h of incubation in the present study.

Marino et al. (2010) measured gas production up to 96 h after incubation and reported a gas production about 198 ml for mushroom waste (including whole mushroom). The extent of gas production at 24 h (176 mL) in the mushroom stem in our research is inconsistent with that reported by Marino et al. (2010) (138 mL). This can be attributed to the nature of experiment mushroom wastes. Yousefi (2016) reported a 24-h gas production of 193 mL from mushroom stem, which, despite similar experiment feeds, differences can be explained by variance in sampling, mushroom cultivars, the growth stage of the mushroom, and different experiment rumen fluid. In the present study, compost gas production extent was 64 ml at 24 h after incubation. Gas production extent of intact compost silage (with bed soil) with 7.5 and 15% molasses were found about 54 and 65 mL per g DM, respectively. These values in de-soiled compost silage with 7.5 and 15% of molasses were reported about 56.88 and 64.76 mL/g DM, respectively (Kalvandi et al. 2018). The growth rate of mycelium, cultivar type, and harvest stage at sampling time can be explained these different results between studies in over the world.

Some of factors affecting the gas production data can be related with harvesting time, amounts of soluble and insoluble carbohydrates, the amount and origin of rumen fluid, rumen fluid donor type, rumen fluid donor diet, and rumen fluid collection time (Taghizadeh *et al.* 2013).

In vitro DM disappearance

The results of DMD values and degradability coefficients are presented in Table 3 and Table 4, respectively. DMD values were significantly different at all incubation times for experiment feeds.

	Table 2	In vitro	gas pi	roduction	characteristics	(mL/g)	DM)	of feed	samples	(n=5)	
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In substion time		Feed		D value	SEM	
incubation time	Alfalfa	Stem of mushroom	m Compost		SEAVE	
2	33 ^a	31 ^a	22 ^b	0.0002	4.9	
4	64 ^a	53 ^b	31°	< 0.0001	2.5	
6	85ª	72 ^b	37°	< 0.0001	3.0	
8	103 ^a	99 ^a	40^{b}	< 0.0001	3.2	
12	125 ^b	135 ^a	46 ^c	< 0.0001	3.1	
24	154 ^b	176 ^a	64 [°]	< 0.0001	2.9	
48	179 ^b	207 ^a	89°	< 0.0001	2.9	
72	190 ^b	219ª	102 ^c	< 0.0001	2.7	
96	195 ^b	225ª	109 ^c	< 0.0001	2.8	
120	198 ^b	228ª	112 ^c	< 0.0001	2.7	
A ¹	200	223	114	-	-	
\mathbf{c}^1	0.08	0.07	0.03	-	-	

¹ A: potential gas production (mL g^{-1} DM) and c: fractional rate of gas production (h^{-1}).

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 3 In vitro dry matter degradability (% DM; n=3)

E			Incubat	ion time		
reed	4	8	12	24	48	72
Alfalfa	33.6 ^b	37.4 ^b	45.2 ^b	48.3 ^b	49.3 ^b	53.1 ^b
Stem of mushroom	45.3 ^a	50.9 ^a	56.2ª	70.9 ^a	75.3 ^a	89.4 ^a
Compost	29.6°	31.2°	32.5 ^b	34.3°	44.8 ^c	48.3°
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SEM	0.575	0.567	0.574	0.576	0.574	0.574

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

Table 4 In vitro dry matter degradation characteristics and effective degradation of experiment feeds (% DM)

Feed	DM degr	adation characte	ristics	Ef	Effective degradation			
	a^1	b ²	c ³	0.02	0.05	0.08		
Alfalfa	24.5°	26.9 ^b	0.065 ^a	46.9 ^a	42.3 ^b	39.3 ^b		
Stem of mushroom	39.7 ^a	53.5ª	0.030 ^{ab}	71.9 ^a	59.9ª	54.5ª		
Compost	27.9 ^b	48.0 ^a	0.008 ^b	41.6 ^c	34.5°	32.3°		
P-value	< 0.0001	0.0006	0.1089	< 0.0001	< 0.0001	< 0.0001		
SEM	0.545	2.467	0.015	0.219	0.266	0.215		

^{1, 2, 3} Constants in the equation $P = a + b (1-e^{-ct})$

Where:

P: level of degradation at time t.

a: readily soluble fraction.

b: insoluble fraction but degradable in rumen.

c: rate of degradation of b per hour.

Effective degradation (k=0.02; 0.05; 0.08)= effective degradability calculated with outflow rates of 2, 5, and 8%.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

This value for mushroom stem, alfalfa, and compost treatments at 48 h of incubation were 75.3, 49.3, and 44.8, respectively (based on DM %). High correlation was observed between DM and CP disappearance both *in vitro* and *in situ*, with alfalfa DMD levels of 50.4 and 48.4 *in vitro* and *in situ*, respectively (Paya *et al.* 2008). Another study reported a DMD value about 48.56 at 48 h for alfalfa using the *in situ* method (Taghizadeh *et al.* 2013). In a similar time, a DMD value of 49.33% was observed for alfalfa in the current study.

To compare our results with other diet ingredients in livestock diets, *in situ* evaluation of DMD levels for alfalfa, red clover, and wheat straw led to values of 37, 37.5, and 72.1, respectively, after 48 h of incubation (Paya *et al.* 2008). A comparison between the degradability of wheat straw (72.5) and mushroom stem (75.3) indicates rather similar DM degradability at 48 h of incubation.

However, the degradability process of these two feeds revealed a faster process for the mushroom stem than the wheat straw.

It should be noted that results from other studies for DMD of wheat straw and mushroom stem compost are not available. Nonetheless, an *in situ* study on rice straw compost reported DMD levels of 607, 675, and 689 g/kg at 24, 48, and 72 h of incubation, respectively (Kim *et al.* 2011). These differences can be due to differences in chemical composition of rice straw compost and its difference with that of wheat straw.

Feed	OMD	ME	NE ₁	SCFA
Alfalfa	53.9 ^b	7.15 ^b	4.02 ^b	0.68 ^b
Stem of mushroom	59.4ª	8.01 ^a	4.82 ^a	0.78^{a}
Compost	59.6ª	4.7 ^c	2.29°	0.28 ^c
P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
SEM	0.350	0.438	0.438	0.437

Table 5 Evaluated OMD, ME, NE1 and SCFA by in vitro gas production results

OMD: organic matter digestibility (%); ME: metabolizable energy (MJ kg⁻¹ DM); NE_i: net energy of lactation (MJ kg⁻¹ DM) and SCFA: short chain fatty acid (mmol 200 mg⁻¹ DM).

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means

Due to the low ADF content in the compost, the DM degradability of the compost was expected to be higher than that obtained, but due to the high amount of crude ash in (Table 1), the DM degradability of the compost was lower than the other experiment feeds.

The outflow rates of 0.02, 0.05, and 0.08 per hour reduced effective DM degradability with increasing exit rates. Even so, mushroom stem had the highest effective degradability at all passage speeds indicating that mushroom stem can also be used in high-yield livestock diets with high levels of DM intake.

Estimated metabolizable energy, net energy of lactation, digestible organic matter, and short-chain fatty acids

Estimated parameters of gas production for instance ME, NE_1 , OMD, and SCFAs are shown in Table 5. The compost contained the highest OMD (based on DM %) with no statistical differences despite the numerical differences in this parameter between the compost and mushroom stem. Alfalfa showed the least amount of OMD compared to the other experiment feeds.

The calculated ME (MJ/kg DM) was significantly different among the three feeds, with values of 8.01, 7.15, and 4.70 for the mushroom stem, alfalfa, and compost, respectively. ME values of 9.7 and 7.7 MJ/kg DM were found for alfalfa and mushroom stem, respectively (Yousefi, 2016) and 8.00 MJ/kg DM for mushroom residue (Marino *et al.* 2010). Since some values such as gas production at 24 h plus CP and fat were used in ME prediction formula, a change in each of these elements can be expected differences result in calculated ME.

 NE_1 calculated for the stem, alfalfa, and compost contained 4.82, 4.02, and 2.29 MJ/kg DM, respectively. A value of 15.5 MJ obtained for full flowering alfalfa with 15 percent protein in dairy NRC (2001) is higher than that of this research. According to the NRC (2001) report on dairy cows, NDF is on average less digestible compared to nonfibrous carbohydrates; therefore, NDF concentration in feeds or diets is negatively correlated with the energy concentration. The NDF chemical composition (cellulose, hemicellulose, and lignin ratios) affects NDF digestibility. Thus, feeds and diets with similar NDF concentrations do not necessarily have the same NE₁ concentrations, and some feeds or diets with high NDF may contain higher NE₁ than those with lower NDF concentrations.

The calculated SCFAs (mmol/200 mg DM) were significantly different between the three treatments of mushroom stem (0.78), alfalfa (0.68), and compost (0.28).

CONCLUSION

Evaluation of nutritional compositions of white mushroom stem, ME and NE_1 values, DOM levels, and DM and CP digestibility, demonstrates that this byproduct has higher nutritional value than late flowering alfalfa. Hence, it can be used in ruminant diet at a lower price than alfalfa due to its availability in different parts of the country.

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