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Investigation of the potential use of spent *Pleurotus ostreatus* (wild type, originated from Picuris Pueblo, New Mexico) substrate in ruminant feed

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SUMMARY. We tested the hypothesis of the use of spent mushroom substrate from *pleurotus ostreatus* mushrooms grown on salt cedar wood as well as the substrate mixed with corn silage for use in animal feeding.

We confirmed the results of previous investigations suggesting that waste salt cedar biomass can be used as a substrate for mycelial growth and fruiting of the edible mushroom *Pleurotus ostreatus*, and examined alterations of the chemical composition, mineral content and level of digestibility of the substrate during mushroom growth. The most significant modification found was in the nitrogen (N) content, which was reduced from 0.39% in the beginning of the life cycle of mushrooms to 0.19% at the end of the fruiting period. The content of Neutral Detergent Fiber (NDF) and hemicellulose (the more easily degradable lignocellulose complex) were lower at the end of growing cycle by 2.62 - 3.08%, consistent with the known activity of *P. ostreatus* enzyme complex (cellulase, hemicellulase, celobiase, ligninase etc). In contrast, the quantity of Acid Detergent Fiber (ADF), Acid Detergent Lignin (ADL) and cellulose (the less-easily degradable complex) was not changed significantly during the mushroom growing cycle. The digestibility of the substrate dry matter after the end of the mushroom growing cycle was lower (8.94%) than the digestibility immediately after inoculation of the substrate (10.84%).

These data suggest only modest possibilities for utilizing spent *P. ostreatus* substrate produced by salt cedar sawdust directly in animal feeding. However, we have established the hypothesis for using spent mushroom substrate as a component of silage production together with ground corn grain.

1. Material and methodology

Corn (maize) grain (Food and Agricultural Organization - FAO group 600) was harvested and ground by machinery. Dry matter content in corn grain at the time of harvest was about 70%. The particle size of collected corn grain was between 0.1 and 2 mm (mesh 4 mm).

Spent *P. ostreatus* substrate originated from Picuris Pueblo, New Mexico, and cultivated at the Sustainable Communities/ZERI laboratory in Santa Fe. Dry matter content in the substrate at the time of silage preparation was 52.7%. The spent substrate particles were very heterogeneous in size (diameter 1-7 mm, length 5-100 mm).

Silage was prepared on November 4, 2005. Ground corn grain and spent substrate were mixed in four different proportions (Table 1), and placed in plastic buckets. (volume V=3kg).

Table 1. Plan of experiment

Silage components	Ratio high moisture ground corn and spent ***P. ostreatus*** substrate, %			
	I	II	III	IV
High moisture ground corn	100	90	80	70
Spent ***P. ostreatus*** substrate	0	10	20	30

By applying pressure by hand on the mixture in the dishes, we tried to expel as much air as possible. Bucket contents were covered by plastic black sheeting (0.2 mm) and plastic caps. Buckets were incubated at 20^{o} C for 28 days and then placed at room temperatures (16-20^{o} C) for 15 days. After the 43-day total period, on December 17, 2005, the buckets were opened, and samples of the silage (0.6 kg per sample) were removed and stored in plastic bags at -18^{o}C until the beginning of the laboratory investigation. Laboratory tests included the following examinations:

- Chemical composition of the silage
- Content of micro- and macro- elements
- Microbiological analyzing
- Quality of the silage

2. Results of investigation

2.1. Chemical composition of the silage

The chemical composition of the raw materials used in the investigation (ground corn grain and spent substrate) and silage obtained in the experiment is presented in Table 2. Figures present significant differences in the chemical composition of the used components in all aspects. Spent *P. ostreatus* substrate had significantly higher ash contents as well as lignocellulose fractions (ADL, ADF, ADL, and cellulose). However, protein content was considerably lower. Chemical composition of the silages (I-IV) differed depending on the proportion of the silage components. The higher the proportion of the spent substrate in silage (from 10% to 30%), the higher were the lignocellulose fractions and the lower the protein content.

Table 2. Chemical composition, % dry matter (DM)

Item	High moisture ground corn	Spent ***P. ostreatus*** **substrate**	Silage			
			I	II	III	IV
Dry matter	**76.41**	**52.07**	65.88	63.36	59.07	57.19
Ash	**1.71**	**13.12**	1.47	2.14	2.82	3.55
Protein	**8.51**	**3.81**	8.70	8.46	8.07	7.81
Fat	**3.81**	**0.65**	3.28	3.07	2.76	2.55
NDF	**17.88**	**65.47**	15.41	18.16	20.65	23.64
ADF	**3.17**	**52.98**	2.73	5.70	8.78	11.99
ADL	**0.43**	**14.19**	0.37	1.19	2.05	2.94
Hemicellulose	**14.71**	**12.51**	12.68	12.46	11.87	11.65
Cellulose	**2.74**	**30.79**	2.36	4.02	5.74	7.54

Organoleptic observation confirmed the specific smell of the silage in all experimental trials (I – IV). In trials III and IV (20 and 30% spent substrate), a weak smell of the spent oyster mushroom substrate was recognized. This specific smell can influence lower consumption of the silage which content 20% and 30% spent substrata.

2.2. Content of macro and micro elements in silage

Results of micro- and macro-elemental analysis of the ground corn grain, spent substrate and test trials of silage are presented in the Table 3. Micro- and macro-element contents of the investigated materials were significantly different. *P. ostreatus* spent substrate contains a significantly higher quantity of Calcium compared to corn grain (0.08%:1.12%). Significantly higher contents of Sodium, Copper, Manganese, Zinc, and Cobalt in spent oyster mushroom substrate were also found, and the quantity of the Lead (Pb) in spent substrate was also significantly higher (15:40 ppm). Lower values for Phosphorus, Potassium, and Iron were found in spent substrate compared to the content of these minerals in corn grain, but contents of Magnesium, Chlorine and Cadmium were almost the same. Consequently, estimated mineral content in the silage trials were in relation to the portions of the raw materials used in silage preparation.

Table 3. Contents macro/microelements (on the dry matter basis)

Macro/ micro elements	High moisture ground corn	Spent ***P. ostreatus*** substrate	Silage			
			I	II	III	IV
Calcium, %	0.08	1.12	0.08	0.15	0.24	0.32
Phosphorus ,%	0.10	<0.01	0.10	0.09	0.08	0.08
Potasium ,%	0.43	0.16	0.43	0.42	0.39	0.37
Sodium ,%	0.01	0.07	0.01	0.01	0.01	0.02
Magnesium,%	0.14	0.20	0.14	0.15	0.15	0.16
Chlorine,%	<0.01	<0.01	<0.01	<0.01	>0.01	0.01
Sulfur,%	0.19	0.27	0.19	0.19	0.20	0.21
Copper, ppm[1]	5	11	5	5	6	6.5
Manganese, ppm	10	103	10	16	24	31
Zinc, ppm	20	65	20	23	27	30
Iron, ppm	345	225	345	336	327	317
Cobalt, ppm	<0.5	2.5	<0.5	0.6	0.8	1
Lead, ppm	15	40	15	17	19	21
Cadmium, ppm	<05	0.5	<05	<05	0.5	>.5

[1] **parts per million**

2.3. Microbiological analysis

Total number of micro organisms in the investigated silage was between 10,000 g^{-1} (silages I-III) to 150,000 g^{-1} (silage IV). Total number of the micro fungi was between 20 g^{-1} (silage I) to 300 g^{-1} (silage II). The total number of yeast was less than 10,000 g^{-1}. A greater number of yeast cells was found in silage samples III and IV.

However, it is important to point out that the estimated number of micro organisms in all silage samples does not exceed recommended limits for all kinds of animal feed (Table 4). The highest number of micro organisms and yeast cells was found in the samples of silage with the highest proportion of *P. ostreatus* spent substrate. However no pathogenic micro organisms (*Salmonella, Stafllicoccus, Proteus, E. coli* and *Clostridium*) were found in any of the silages, but a few molds species (1-2) were found (Table 5).

Tests for mycotoxin presence (by low layer chromatography) were negative. In particular, no type of mycotoxin (Aflatoxin B_1, Ohratoxin A, Zearalenon, Diacetoxiscirpenol -DAS and T_2- toxin) was detected, confirming that the population of mold species recognized in the silage samples was very small and could not produce significant quantities of the mycotoxins recognisable by routine, applied methodology.

Table 4. Microbiological analyses (per 1 g of sample)

Item	Silage			
	I	II	III	IV
Total number of micro organisms	10 000	80 000	10 000	150 000
Total number of molds and micro fungi	20	300	100	40
Total number of yeast cells	<100	<100	<1 000	<10 000

Table 5. Detected mold species

Species	Silage			
	I	II	III	IV
Penicillium brevicompactum		+	+	+
Penicillium funiculosum	+			
Penicillium variabile	+			
Penicillium echinulatum		+	+	

2.4. Silage quality

Silage pH values (degree of acidity) decreased slightly as the proportion of the spent substrate in the silage increased (Table 6), but did not fall below 3.5, even in sample IV. The predominant fatty acid, belonging to the group of evaporated fatty acid, was lactic acid. Estimated quantities of this acid in the silages were between 3.16 and 3.77%. The quantity of acetic acid was 4-5 times lower than the quantity of lactic acid (0.61-0.83%). Butyric acid was not detected. The proportion of ammonium nitrogen (NH_3–N) was within the optimal range (0.071-0.083% DM or 5.24-6.4% of the total nitrogen). The quantity of the NH_3–N was slightly higher in samples III and IV. Those results demonstrate that in all silage samples optimal fermentation conditions existed.

Table 6 Silage qulaity

Parameter	I	II	III	IV
pH	3.77	3.79	3.64	3.59
Lactic acid,% DM	3.16	3.23	3.77	3.55
Acetic acid ,% DM	0.61	0.68	0.83	0.69
Butyric acid,% DM	0.00	0.00	0.00	0.00
NH_3-N,% DM	0.074	0.071	0.078	0.080
NH_3-N,% TN	5.31	5.24	6.04	6.40
Digestibly, % DM	86.80	85.61	80.69	77.91

Dry matter digestibility of the silage decreased with increasing quantity of the spent oyster mushroom substrate in the silage. Digestibility of the silage decreased from 86.80% (value for the silage prepared based on the pure ground corn grain) to 77.91% in silage sample IV (70% corn grain; 30% spent substrate). These results can be explained by the increased portion of the spent substrate and the consequent increase in the less-easily degradable lignocellulose fractions.

Conclusion

Adding spent *P. ostreatus* substrate based on salt cedar into the ground corn grain (proportions from 10 to 30%), influenced chemical exchange in the silage. The most important differences were in NDF, ADF, hemicellulose, cellulose and lignin quantity.

Chemical laboratory tests showed that substrate contained higher concentrations of Calcium, Sodium, Sulfur, Copper, Manganese, Zinc, Cobalt, and Lead. Lower values for the content of minerals in substrate in comparison with ground grain corn were found for Phosphorus, Potassium and Iron. Almost identical values in both materials were shown for Magnesium, Chlorine, and Cadmium. Consequently, mineral content in the all silages samples were correlated with the mineral content of the components.

Microbiological analyses confirmed that the presence of micro organisms, molds, and yeast in the silage were in the range of the tolerances for animal feed, and mycotoxins [Aflatoxin B1, Ohratoxin A, Zearalenon, Trihoteceni (DAS and T_2 toxin)] were not detected. Contents of evaporated fatty acids in the silages, pH values and NH3-N were in the range of the values characteristic for the silage of very good quality. With increasing the content of the spent substrate in the silage, digestibility of the silage decreased, which is, besides the taste and smell, the primary limiting factor for incorporating this material in the routine daily cattle feed.

Use of spent oyster substrate in animal feeding can be recommended for the meal with lower content of ADF and NDF, eventually. Namely, spent substrata content high quantity of the ADF and NDF (Table 2) and because of that this is complementary feed with corn grain which is characterized by low fiber quantity. Under the extensive feeding conditions of the cattle with lower genetic potential - (milk production 3-4.000l/per year/per cattle, daily gain in the weight up to the 1000g/day), this feeding opportunity can be taken into consideration as well. However, this conclusion must be tested with further feeding experiments.

Based on our results, it is possible to conclude that spent oyster mushroom substrate made by salt cedar sawdust (moisture content about 25%), can be used as a component for wet corn grain (moisture content about 75%) silage production. Optimal proportions were 80% of wet corn grain: 20% of spent substrate.

However spent substrate must be milled very well, which consequently means that sawdust for substrate production MUST be small (ie. nickel sized) and very well milled.

Such silage, if it is kept under adequate conditions, has parameters listed below:

- optimal acidity and lactic acid content
- absence of pathogens, microorganisms, fungi and molds which produced mycotoxins,
- good digestibility (>80%)

Such silage CAN be recommended in beef for fattening and in milk cows in quantities up to 5 kg of feed per animal per day. This silage quantity would be mixed with other feed (protein, carbohydrate and mineral – vitamins components, silage or lucerne hay and dry grass, etc.) and offered to the animals 3 times per day: morning – noon- evening. However it must be pointed out that the animal must get used to that kind of feed, incrementally.

For the first three days, silage must be given in smaller portions, i.e. 1-2 kg per animal per day; from the 4th to 6th day, 3-4 kg per animal per day; after 7 days silage quantity can reach the level of 4-5 kg per animal per day. If the animal cannot consume the offered quantity, we recommend that the quantity of feed be lowered.

Recommendation

Further investigations should be directed towards testing silage production based on spent substrate in combination with dry corn grain with the addition of non-protein nitrogen compounds (e.g., urea+zeolite), bacterial enzyme inoculants (lactic acid bacteria with addition of specific enzyme), or other additives to improve silage quality as well as the taste and smell of this kind of alternative animal feed. An advantage of silage production with dry corn grain instead of the fresh ground corn grain used in our experiments is that the former way of silage production can be an all-year process. Using fresh corn grain is limited only to the corn harvesting season. Moreover, the moisture content of the spent oyster mushroom substrate can compensate for the lack of moisture of the dry corn grain, and together both components can provide optimal moisture content for fermentation of corn carbohydrates, consequently improving silage quality. These tests can also be expanded to areas that use other feed types besides corn such as alfalfa.

However, in order to precisely define the potential utility of spent oyster mushroom substrate in the routine livestock feeding, pure or as a component of the silage, it is necessary to conduct biological tests where reactions of the animals (daily yield, quantity and quality of the milk, reproductivity, physiological parameter, etc.) can be evaluated. The economic effect of the specific feeding program must be taken into consideration as well.