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Potential for use of Spent Substrate of Pleurotus Mushrooms Grown on Urban Waste as Feed for Dairy Cattle

Abstract

Mushroom wastes are available in high volumes, with 5 million tons of spent mushroom substrate (SMS) being disposed of globally every year. Due to this high availability, various forms of SMS have been researched for their use as alternative animal feeds. Additionally, experimental techniques can be used to grow certain mushroom species, such as oyster mushrooms (*Pleurotus* sp.) on various lignocellulosic waste materials. Therefore, the SMS from *Pleurotus* sp. grown on these waste materials may offer a promising conversion from a waste material to a low-cost, nutritionally sufficient feed. However, little research has been done to determine if feeds from *Pleurotus* SMS specifically grown on urban waste substrates offer the same benefits. Given rising awareness on circularity and urban self-sufficiency, growing mushrooms on urban waste is a promising solution which should be investigated. This paper assesses the feasibility of using SMS from golden oyster mushrooms (*Pleurotus citrinopileatus*) grown on urban waste as dairy cattle feed, comparing substrate ratios to determine which would result in the most desirable protein and fiber contents. SMS from three experimental substrates of cardboard and spent coffee grounds (SCG) were compared to traditional dairy cattle feeds. Treatments 2 and 3 were found to be suitable for use as additives to traditional feeds in small replacement amounts. However, both treatments also had high fiber content, which may affect practicality of use as feeds.

Introduction

The cultivated mushroom industry is growing rapidly, with global production of edible mushrooms having increased 30% since 1978[1]. In Canada, the mushroom market is dominated by *Agaricus* sp. (including button mushrooms – *Agaricus bisporus*), which account for 98% of production; other "specialty" mushrooms such as *Pleurotus* sp. account for the remaining 2%[2]. Valued at 63 billion USD in 2013, the global mushroom market is likely to continue growing, given the rising demand for non-animal proteins[3]. The rise in mushroom consumption is promising from an environmental perspective due to the intense resource use associated with animal protein; however, environmental issues also arise in the form of spent mushroom substrate (SMS), the material left behind after mushroom fruiting bodies have been harvested[4,5,6].

The large quantity of SMS left over, approximately 5 kg for every 1 kg of harvested mushroom in *A. bisporus* production, is seen by mushroom producers as waste, resulting in an astonishing 5 million tons of SMS solid waste being disposed of annually[7,8]. However, this SMS has several documented alternative uses, primarily being used as fertilizer. Despite its effectiveness as a fertilizer, the storage and transportation costs associated with disposal of SMS by field application incurs such high costs that it can be less economically viable than chemical fertilizers[6]. Additionally, with growing awareness on the benefits of circularity, SMS uses which can replace raw inputs by being returned into a cycle, such as animal feed, are more desirable[9].

Typical agricultural products used for cattle feed contain high amounts of nutrients, but are difficult to digest and are therefore inefficient in their conversion of a raw agricultural product to usable energy[7]. There are also issues with importation of more nutritionally valuable feeds; the European Union is aiming to reduce its high import dependency (70%) on soy-based, protein-rich animal feed[9]. Therefore, alternatives are needed for local production of high quality, protein-rich animal feeds which are high in nutrients, easy to digest, and economically viable. In this paper we investigated the possibility of using SMS from mushrooms grown on locally generated urban waste—cardboard and spent coffee grounds (SCG)—as dairy cattle feed.

Literature Review

Traditional ruminant feeds include straw and other agricultural residues; however, these feeds have low available energy, protein, and mineral content because digestion is impeded by high quantities of hard-to-digest cell wall components such as cellulose, hemicellulose, and lignin[10]. Delignification of straw through chemical treatments can increase nutritional value; however, these processes are both economically and environmentally undesirable[11].

A good alternative is delignification through biological processing of raw materials; some fungi are very efficient decomposers of these cell wall components, especially of lignin[12], and therefore can be used for biological delignification. An added benefit of biological delignification using mushrooms is the production of a valuable food source for humans (harvested mushrooms).

A preliminary report by Weiss et al. (1980) discussed the initial results of their ruminant feed study, which incorporated *A. bisporus* mushroom waste in the form of SMS and mushroom stumps[13]. In this study, mushroom wastes ensiled with hay and corn showed increases in crude protein (CP), calcium, and acid detergent fiber (ADF). However, CP content decreased in treatments without corn, implying that the increased protein content could be more attributable to corn than mushrooms. Nonetheless, the analysis showed that mushroom supplemented diets could meet nutrient requirements for a wide range of ruminants, subject to confirmation with a metabolism study. One core issue found by the authors is the low dry matter (DM) content of the *A. Bisporus* SMS, which makes transport of this SMS unnecessarily costly due to high moisture content.

The nutritional consistency of SMS is another issue to consider, as a standard diet must be maintained for cattle; unfortunately, the authors found the primary hurdle in feed development to be the inconsistency of *A. bisporus* mushroom waste-based livestock feed, which makes it difficult to formulate a standard diet. However, this statement is not consistent with general knowledge as *A. bisporus* cultivation is highly standardized and has quite low variability compared to other mushroom species which are less commonly cultivated[14]. Additionally, using the results of metabolism studies, standard diet formulations can

be developed which help both the farmer (by lowering feed costs) and the mushroom producers (by aiding in waste disposal).

A paper by Wilson et al.[15] discusses the results of a lamb metabolism trial using ensiled hay, corn, and *A. bisporus* mushroom waste feed. This study tested three diets containing 10% hay, 15% corn, and 75% *A. bisporus* mushroom waste, the last component being varied between trials with either all compost, all stumps, or a half and half mix of the two. The results of the feeding trial showed that lambs experienced a reduced rate of growth when consuming feed with mushroom wastes, compared to a standard diet. The low energy value of SMS made it ineffective in meeting the nutritional demands of young animals, however the authors noted that SMS could be incorporated at 25-33% in diets of mature animals, who have lower nutritional requirements, and could be included at levels less than 15% in the diets of growing animals. The results of this study may discourage the search for a suitable, mushroom-based livestock feed, but expanding beyond *A. bisporus* waste to other mushroom species may provide different results.

Pleurotus sp., commonly known as oyster mushrooms, are the second most cultivated mushroom worldwide[16], accounting for 27% of global mushroom production[17]. Oyster mushrooms are well known because they are easy to grow, highly nutritious, and can be grown on a wide variety of agricultural wastes although with varying yield rates[7]. *Pleurotus* sp. are high in protein (15-35% on a dry weight basis) and vitamins B and C, and can be productively grown on a huge variety of lignocellulosic compounds, including industry waste products such as pulp sludge, coffee residues, agave waste, and soy pulp[12].

A 1998 feeding trial by Adamović et al.[18] studied the use of SMS from *P. ostreatus* grown on wheat straw as a cattle feed. They found that cell-wall components of the straw, especially lignin and cellulose, decreased during incubation due to degradation by *P. ostreatus* enzymes, corresponding with an increase in protein content and digestibility of the substrates. However, despite a theoretical improvement in feed quality, the feeding trial showed that average daily gains were smaller in both groups consuming *P. ostreatus* SMS compared to a control group eating their regular feed. This result can be attributed to low palatability of the SMS feed since during the trial, the cattle rejected SMS unless it was mixed with silage, refusing to consume anything with more than 17% SMS as a portion of total feed dry matter (lowered from the original trial goal of 20%). The group consuming 10% SMS had only 10 g less gain than the control group, compared to 60 g less for the group consuming 17% SMS. It is difficult to determine how much of this reduction is due to reduced feed intake, and how much is due to the quality of the feed itself; if some solution could increase the willingness of cows to eat the SMS feed, P. *ostreatus* SMS could be a valuable feed additive to increase protein intake for livestock.

While the use of agricultural wastes such as *Pleurotus* sp. substrate has been extensively studied, there is far less published academic information regarding the use of urban wastes as substrates. However, one student research paper conducted at McGill University studied the feasibility of growing oyster mushrooms on SCG and either cardboard or coffee filter paper, finding that using SCG as a substrate resulted in satisfactory fruiting results, and also reduced both energy costs and urban generated waste compared to typical commercial substrates[19]. One issue found in this research was the increased risk of contamination by what was referred to as "green mold" when using SCG and cardboard substrates, compared to SCG and filter paper.

The author speculated that this may be a result of introduced contaminants from the cardboard, as the filter paper is covered until use as a substrate. Green mold does not refer to a specific species, so it is unclear what organism the author is referring to. However, common contaminants of *P. ostreatus* mushrooms include competitors such as *Pseudomonas*, *Bacilli*, and coliform bacteria, and undesirable fungi such as *Trichoderma, Penicillium*, and *Aspergillus*, all of which have inhibited growth in more alkaline substrates[20]. Therefore, the acidity of SCG may also have raised the contamination risk through lowering of the substrate pH to the point of increased risk of contamination.

Use of SCG in substrates may increase contamination risk, but it also increases protein content which helps to increase yields; therefore, a balance of SCG content must be found[12]. In a previous study conducted by the authors, this balance was tested by growing grey oyster mushrooms (*Pleurotus ostreatus var. columbinus*) on five substrates with coffee contents of 0%, 25%, 50%, 75%, and 100% by wet weight, with cardboard composing the rest of the substrate. The treatments with 25% and 50% coffee were the best performing, with treatments having higher than 50% coffee failing, and the 0% coffee treatment performing poorly.

The lack of further academic research on *Pleurotus* sp. cultivation on urban wastes is indicative of a gap between academic and general knowledge. Information is widely available online regarding the efficacy of growing oyster mushrooms on cardboard and SCG, as both products are widely available in urban settings; however, little academic research has been done to support these claims. Given growing awareness on the need for increased urban waste redirection through the circular economy, in which resources are recovered and reapplied in different cycles[21], this research gap should be rectified. This paper will contribute by growing oyster mushrooms on urban waste substrates (cardboard and coffee) and assessing the suitability of the resulting SMS for use as cattle feed.

Materials and Methodology

Materials

All substrates used were diverted from the waste streams of local Montreal businesses. Cardboard was collected from a recycling bin behind a grocery store, with only cardboard that was clean and without visible glue or ink being selected. Coffee was collected from Café Névé with help from employees, who placed the SCGs in a closed container after brewing for collection.

Mushroom spawn was purchased in 1 kg quantity from Mycoboutique (Montreal, QC). The strain used was *Pleurotus citrinopileatus* (Yellow Oyster, or Golden Oyster).

A shotgun fruiting chamber (SGFC) was constructed following instructions from FreshCap Mushrooms[22], shown in Figure 1. Once constructed, the SGFC was propped up on cups to ensure it was high enough off the ground for proper airflow to be established, according to recommendations on an online SGFC forum.

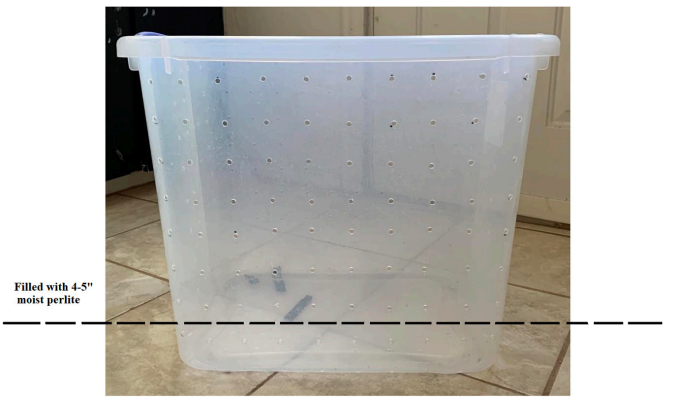

Figure 1. SGFC chamber pictured before being filled with moist perlite and propped up.

The mushrooms were grown in #4T polypropylene bags with 0.2 micron filter patches (Mycoboutique, Montreal, QC). Sterilization of tools and surfaces was done with 70% isopropyl alcohol. A generic kitchen scale with ±1 g accuracy was used to weigh the substrates and spawn.

Methodology

The main goal of this study was to find which substrate ratio of cardboard to SCG produced the best SMS for dairy cattle feed after oyster mushroom cultivation. The protein and fiber content of the SMS were used as proxies for nutritional quality and digestibility, with higher protein and lower fiber being desirable. I hypothesize that:

1. Higher SCG content in substrate will result in lower fiber content.
2. Higher SCG content in substrate will result in higher protein content.

In order to test these hypotheses, substrate samples will be taken both before and after mushroom harvest (section 4). These samples will then be tested to allow for full comparison to recommended dairy cattle diets (section 5), and statistical analysis will be performed, using these results, to test the hypotheses (section 6). Based on the results of previous research, three substrate treatments were devised, all with a 20% spawn rate, equal total substrate weight, and lower than 50% coffee content. Three replicates were prepared for each treatment. The substrate ratios for each of the three treatments is displayed in table 1 below.

Table 1. Summary of substrate composition for each treatment.

Treatment	Cardboard (g)	Coffee (g)	Spawn (g)
1	615	0	123
2	492	123	123
3	369	246	123

1. Preparation

Cardboard was cleaned using hot water pasteurization[23]. Boxes were cut into large pieces, placed in a large sturdy plastic storage container, and soaked in boiling water for two hours. Then, pieces were drained, stacked, and covered. Collected SCG were pasteurized in the brewing process and used within 24 hours of collection; therefore, no sterilization was performed. After substrate preparation and material collection, all surfaces were sterilized using 70% isopropyl alcohol. After this had completely evaporated, inoculation began.

2. Inoculation Procedure

Three bags were prepared for each treatment, for a total of nine inoculated bags. Cardboard was torn into small pieces, then layered in the grow bags with coffee and/or spawn[23]. The bags were then sealed with zip ties, placed in a dark room out of direct light, and left to colonize. After 21 days, the bags were fully colonized, and fruiting was initiated.

3. Fruiting Procedure

Bags were cut open to sample substrate (section 4), then firmly sealed with tape. About halfway down the front of the bag, a 1 in. incision was made to allow fruiting[12]. The bags were then placed in the SGFC and misted 3-6 times a day. Fruiting time varied greatly for each bag; the first fruits were harvested 12 days after being placed in the SGFC, compared to 38 days for the last fruits.

4. Sampling

Pre-fruiting sampling was very conservative, as too much disturbance of the substrate could increase contamination risk[12]. Six samples were taken from various locations in each bag, for a total sampled mass of approximately 10 g per bag. After samples were taken, they were placed in a Ziploc bag, labelled, and frozen.

The second round of samples were taken after one flush of mushrooms had been harvested. This post-fruiting sampling was done by cutting the bag open and mixing up its contents in a large bowl. Then 200 g samples were taken in small, random increments from the bowl, placed into a Ziploc bag, labelled, and frozen.

5. Testing

Samples were sent to Agrianalyse (Sherbrooke, Quebec) for analysis. In order to compare the nutritional content of SMS feed to traditional feeds, the samples were tested for crude protein, moisture, neutral-detergent fibre (NDF), and mineral content. NDF content was chosen as it is a measure of cell wall content and, therefore, can be used to determine fiber content and digestibility[24], as well as being a predictor of voluntary intake of feed[25].

6. Statistical Analysis

Results were imported to Excel and then checked for normality using the Shapiro-Wilke test[26]. One-way ANOVA tests were performed on normal data sets to assess statistical significance between the means of the three treatment groups[27]. An independent two sample t-test was also performed to determine if there was a statistically significant difference between two treatment means[28].

Results

Change in NDF content as a result of mushroom digestion was calculated from the pre- and post-fruiting values, shown below in Figure 2. No statistically significant difference in mean change in NDF was found when comparing treatments using one-way ANOVA. Samples of post-fruiting substrates were much larger than pre-fruiting samples, which may have influenced the comparison of pre- and post-fruiting results.

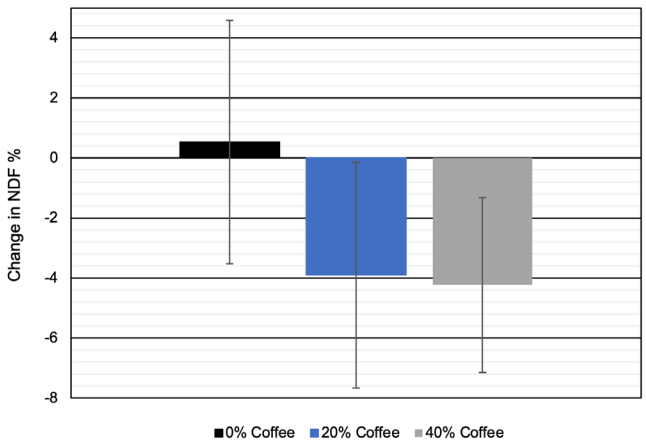

Figure 2. Change in fiber content in SMS as a result of mushroom digestion.

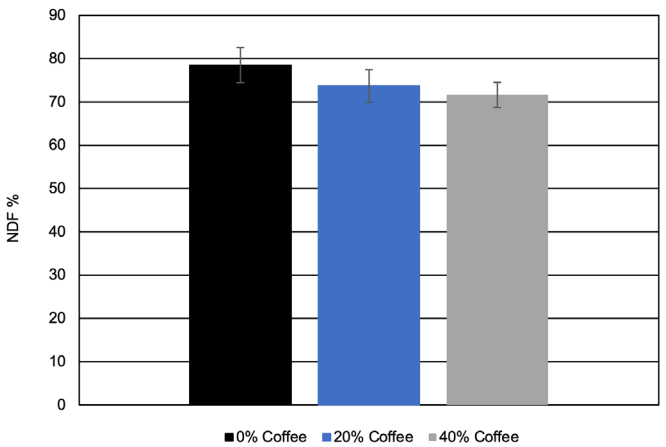

Figure 3. Final (post-fruiting) fiber content of SMS.

The difference in final fiber content between treatments was assessed using both a one-way ANOVA test on all treatments and two-sample t-tests between treatments. High variability in fiber content resulted in a lack of statistical significance from all tests; therefore, the null hypothesis of no difference in mean NDF content between treatments was accepted. Final NDF content for all treatments is shown below in Figure 3.

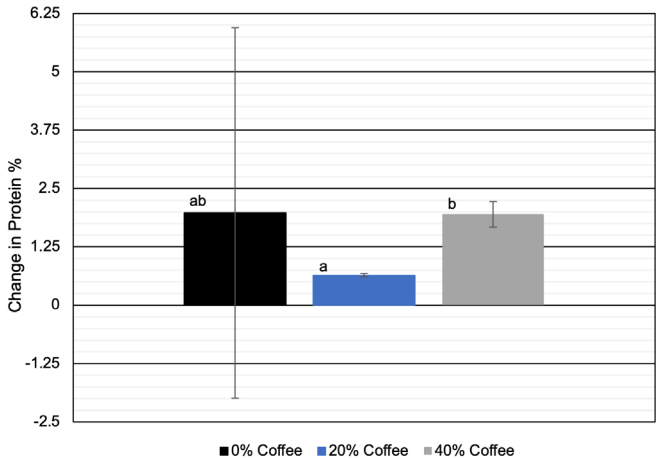

Figure 4. Change in protein content in SMS as a result of mushroom digestion.

Using the pre- and post-fruiting protein values, the change in protein as a result of mushroom digestion was plotted. No statistically significant difference in mean change in protein was found when comparing all treatments using one-way ANOVA; however, the two-sample t-test found a significant difference between treatments 2 and 3 (20% & 40% coffee, respectively), with protein content in treatment 3 increasing by 1.31% more than in treatment 2 (Figure. 4). Samples of post-fruiting substrates were much larger than pre-fruiting samples, which may have influenced the comparison of pre- and post-fruiting results.

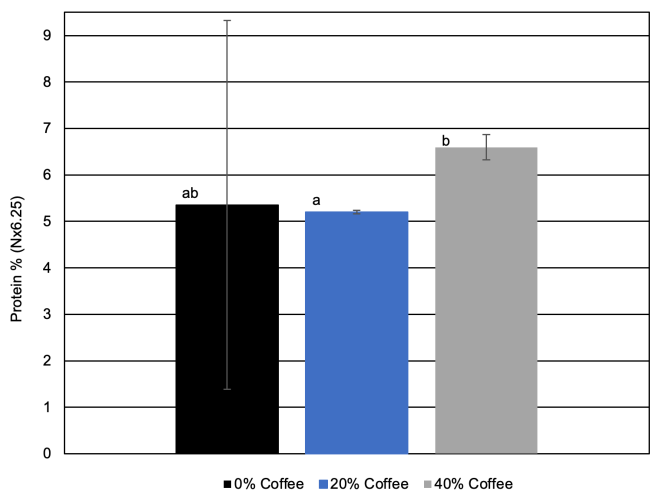

Figure 5. Final (post-fruiting) protein content of SMS.

The difference in final protein content between treatments was plotted, with the one-way ANOVA test on all treatments showing no significant difference between group means, where high variability in treatment 1 was likely a factor. If this variability had resulted from lack of sterile lab space or lack of environmental control, both treatments 2 and 3 would presumably have had comparably high variabilities. However, as both had comparably low variabilities, a more likely cause was that treatment 1 was the only one with 0% SCG, which would affect both the pH and the substrate structure, two major determinants of substrate performance[12]. It is also possible that a random testing error may have contributed to high variability. A two-sample t-test revealed a significant difference between the mean protein content of treatment 2 (5.20% ± 0.0346) and treatment 3 (6.60% ± 0.274), supporting hypothesis 2 (higher SCG content correlates to higher protein content) for these two samples. The results of this analysis are plotted in Figure 5.

Discussion

It is important to note that the sample size of this study was relatively small due to the lack of lab space and high cost of testing. Therefore, the results discussed here should be interpreted with caution before they are confirmed with further studies.

Treatment 1 had the highest variability in protein content, but a slightly higher mean protein content than treatment 2, making it difficult to assess its potential suitability as a feed. The source of variability in measured protein content for treatment 1 was discussed briefly in the previous section but must be further investigated before drawing any definite conclusions regarding the feasibility of using treatment 1 as a feed. Additionally, since this research is focused on the use of urban wastes as cattle feed, and since SCG represent a large, pre-sterilized urban waste feedstock, the treatments containing SCG will be the focus of analysis here. Treatment 3 had the highest final protein content; however, treatment 2 had lower variability, and no significant findings can differentiate NDF content. Therefore both substrate ratios could be considered for use as SMS feed. The results for these treatments are compared to typical dairy cow diets below in Table 2. As predicted, both treatments had much higher protein contents than these typical diets, with over three times the protein required for early lactation cows, demonstrating their potential use as protein supplements. Additionally, both treatments contain around twice the calcium content of a typical diet, so use as a calcium supplement is also possible. It should be noted that the NDF content of both feeds is quite high, more than twice the minimum for dry cows. This is understandable given the high proportion of cardboard present in both treatments, but it unfortunately detracts from the benefits of high protein content due to the inverse correlation between digestibility, voluntary intake, and NDF content.

Table 2. Comparison of typical diet formulations for dairy cows to post-fruiting SMS values for treatments 2 (20% coffee) and 3 (40% coffee).

	Typical diets			SMS values	
	Early	Mid	Late/Dry	20% coffee	40% coffee
Crude Protein (g kg^{-1} DM)	17	14	12	52.00 ± 0.346	65.97 ± 2.74
NDF (%)			33 (min) *	73.70 ± 3.76	71.67 ± 2.91
Calcium (g kg^{-1} DM)	8	6	5	14.6 ± 3.90	12.4 ± 4.40
Phosphorus (g kg^{-1} DM)	4.5	3.5	3.0	0.70 ± 0.140	0.75 ± 0.070
Magnesium (g kg^{-1} DM)	1.8	1.5	1.5	1.03 ± 0.150	1.00 ± 0.170
Sodium (g kg^{-1} DM)	1.8	1.5	1.5	0.50 ± 0.100	0.40 ± 0

All typical diet data from Phillips et al.[10] unless noted

*Erickson & Kalscheur [29]

It is important to consider the application context when discussing use of these treatments as dairy cattle feed. Due to the palatability issue highlighted earlier, the SMS feed should only be used in small quantities (less than 20%), and due to the undesirably high NDF content of the SMS, this issue of palatability may likely be exacerbated. One solution could be use of SMS in a compound feed, in which several ingredients are mixed to supplement nutritional intake of ruminants whose diet consists mainly of forage intake[30]. Compound feeds are typically pelleted; however, another option is pelleting the SMS as a stand-alone supplement without other additions. Pelleted feeds are easier to handle and distribute because they have a reduced dry matter content compared to non-pelleted feeds. During the pelleting process, the moisture content of the feed is reduced, and the feed compressed, resulting in increased bulk density and a corresponding reduction in transportation costs[31]. Pelleted feeds also have their energy content increased compared to the raw input material due to the addition of oil during the pelleting process and the common use of sugarcane molasses as binding agents[10]. Additional advantages of pelleting include enhancement with additives for a number of reasons, such as increased nutritional value and increased palatability[31,32]. On-site pelleting could make transportation of SMS easier and more economical; however, the overhead costs of pelleting must be considered. Pelleting costs vary depending on operation size, but assuming a rough production cost of €101 per tonne of DM for straw pelleting, with major costs being raw materials (66%), various plant operations (21%), and labour (9%)[33], an estimated cost for SMS pelleting can be calculated. Considering SMS as a waste, the only raw material cost will

be transportation of SMS, which will conservatively be estimated at 30% of raw material costs, resulting in a conservative total production cost of €50.3 per tonne of DM ($69.6 CAD). If pelleting occurs at the site of SMS production, transportation costs are negated, and total production cost is reduced to €30.3 per tonne of DM ($41.9 CAD). Considering current feed costs (in $CAD / tonne DM) of $312 for hay, $80 for corn silage, or $687 for performance supplements[34], pelleting SMS for feed is a viable option which should be researched further.

Conclusions

In this study, golden oyster mushrooms (*Pleurotus citrinopileatus*) were grown on three treatment substrates of cardboard and SCG, with the goal of determining which substrate ratio would produce the best SMS for use as dairy cattle feed. The hypothesis was that higher substrate SCG content would result in better SMS for feed, specifically stating that higher SCG content would result in lower fiber content and higher protein content. Hypothesis 1 was rejected due to high variability in treatment results; no statistically significant difference in fiber content between treatments could be proven. Hypothesis 2 was accepted. High variability in treatment 1 was an issue which must be further investigated; however, the t-test showed a statistically significant difference between protein content in treatments 2 and 3, with treatment 3 having 13.97% higher protein content. When comparing treatment results to typical dairy cattle diets, treatments 2 and 3 were found to be suitable for further study due to their high protein and calcium content post-fruiting.

In addition to nutritional advantages offered by SMS feeds, there are potential economic benefits, as the high input cost associated with dairy cattle feed could be greatly reduced through use of a waste product such as SMS to supplement feed. Although pelleting and transportation would have associated costs that may mitigate the economic advantage of using a waste product, it is still possible that this alternative feed would be more cost effective than traditional feeds, especially when considering the nutritional advantages offered. Further study is needed to verify this.

Limitations

The two most pressing issues found in this paper were palatability, high fiber content, and high variability. Palatability can be addressed by pelleting the SMS for use as a feed additive; however, high NDF is an issue which may only be solved by replacing cardboard with another lignocellulosic waste. Variability likely resulted from lack of sterile lab space and environmental control, as well as small sample sizes. Future studies should correct these issues and should also collect and analyse fruiting data as this would enhance discussion and expand the scope of the study.

Outlook

Further research must be done to assess the practical applications of the results presented here. A full feeding trial should be conducted to better determine the palatability and digestibility of pelletized SMS, ideally using both pelleted and non-pelleted SMS feeds; methods such as those used in Adamovic et al.[17] may be of use in designing these trials. An economic analysis should also assess the practicality of implementing these recommendations and may help farmers and mushroom producers pursue these changes, which can be an economic risk.

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