Potential for use of Spent Pleurotus Mushroom Substrate as Feed for Dairy Cattle

Liesl van Wyk, *Bioresource Engineering, McGill University, Montreal, QC*

Supervised by Dr. Grant Clark, *McGill University, Montreal, QC*

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 spp.)* on various lignocellulosic waste materials. Therefore, the SMS from *Pleurotus spp.* grown on these waste materials offers a promising conversion from a waste material to a low-cost, nutritionally sufficient feed. However, little research has been done to determine the viability of SMS specifically grown on recycled substrates, and if these feeds offer the same benefits as SMS grown on traditional substrates. 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 cattle feed. The mushrooms were grown on three experimental substrates of cardboard and spent coffee grounds (SCG), and the nutritional contents of the substrates pre- and post-fruiting were compared to traditional cattle feeds. Treatment 3 (60% cardboard and 40% coffee substrate) was found to have the highest protein, followed by treatment 2, but compared to typical cattle diets both treatments have very high protein content and could be used as an additive to traditional feeds in small replacement amounts. However, both treatments also had high fiber content, which may affect practicality of use as feeds.

Key Terms - Pleurotus, spent mushroom substrate (SMS), cattle feed, spent coffee grounds (SCG)

INTRODUCTION

The mushroom industry is growing rapidly; global production has increased 30% since 1978 and annual consumption more than quadrupling since 1993 (Royse et al., 2017). Valued at 63 billion USD in 2013, the global mushroom market is likely to continue growing, given the rising demand for non-animal proteins (Grimm et al., 2021). 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. After the first flush of mushrooms is produced, most of the cellulose and lignin in the substrate are consumed, making it difficult to reach profitable yields from a second flush (Beyer, 2011). Therefore, after the fruiting stage of mushroom production there is a large quantity of SMS left

over, approximately 5 kg for every 1 kg of harvested mushroom (Finney et al., 2009; Mohd Hanafi et al., 2018), which is seen by mushroom producers as a waste, resulting in an astonishing 5 million tons of SMS solid waste being disposed of annually (Mohd Hanafi et al., 2018). However, this SMS has several documented alternative uses, including energy production, wastewater treatment, and animal feedstock, but primarily by being applied to farmland 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 is less economically viable than chemical fertilizers (Beyer, 2011). Additionally, with growing awareness on the benefits of circularity, SMS uses which can be replace raw inputs by being returned into a cycle, such as animal feed, are more desirable (Grimm and Wösten, 2018).

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 useable energy (Mohd Hanafi et al., 2018). 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 (Grimm and Wösten, 2018). 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 suitability of using SMS from mushrooms grown on locally generated waste - cardboard and spent coffee grounds (SCG) – as an alternative cattle feed, focusing specifically on protein and fiber content as proxies for nutritional quality and digestibility.

LITERATURE REVIEW

Traditional cattle 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 (Phillips, 2004). Delignification of straw through chemical treatment using sodium hydroxide or ammonia is one option to increase its nutritional value, however these commercial processes are both economically and environmentally undesirable (Lucio et al., 2020). One promising alternative is delignification through biological processing of raw materials. Fungi are very efficient decomposers of these cell wall components, especially of lignin (Stamets, 2000), therefore agricultural residues and other forms of lignocellulosic biomass can be effectively delignified through biological processing using mushrooms. The added benefit of biological delignification using mushrooms is that the agricultural waste product is turned into a valuable food source for humans, in the form of harvested mushrooms, as well as for cattle, in the form of SMS.

A preliminary report by Weiss et al. (1980) discussed the initial results of their ruminant feed study, which incorporated mushroom waste (from *A. bisporus* production) in the form of SMS and mushroom stumps. In this study, mushroom wastes were ensiled with hay and corn, and the resulting feed was tested through laboratory nutritional analysis. The ensiled feeds showed increased crude protein (CP), calcium and acid detergent fiber (ADF). The authors noted

that ground corn had to be added in order to aid the fermentation process, and it should be mentioned that, while the treatments which included corn did have increases of approximately 1% in CP levels on average, the treatments without corn actually had decreases in CP content. This means the increase in protein content cannot be attributed to the effect of mushroom waste fermentation, as it could be more attributable to the effect of corn silage. Nonetheless, the values obtained following laboratory analysis showed that mushroom supplemented diets could meet the nutrient requirements for a wide range of ruminants, however these results were subject to confirmation with a metabolism study, as many factors outside of nutritional value can impact the usefulness of a feed alternative, and the results of this study were not included in the preliminary report. While no strong conclusions can be drawn from this paper, it still provides valuable information regarding potential problems in thee use of mushroom waste in livestock feed, some of which were explicitly discussed by the authors. One core issue is the dry matter (DM) content of the SMS, which affects the cost of transportation, as well as storage method and cost. Though not as high as that of mushrooms, SMS still has a very high moisture content at approximately 20-25% DM (Adamović et al., 1998), compared to around 35% for standard ensiled feeds (Weiss, 1980) and 90% DM for air-dried feeds (Reiling, 2011), making transport unnecessarily costly. The nutritional consistency of SMS is another issue to consider, as a standard diet must be maintained for the cattle. Due to the highly variable nature of mushroom production, the nutritional value of the SMS may also vary significantly, making it hard to estimate feed portions. The authors found that the primary hurdle in feed development was the inherent inconsistency of mushroom waste-based livestock feed, which makes it difficult to formulate a standard diet. However, using the results of metabolism studies, in conjunction with farmers, standard diet formulations can be developed which help both the farmer (by lowering feed costs) and the mushroom producers (by aiding in waste disposal). Following the recommendations of the authors, it is clear that assessing the economics of on-site treatment to increase DM and improve nutritional consistency is of utmost importance, compared to transport of raw SMS, and that a feeding trial must be done in conjunction to confirm the accuracy of the results.

While Weiss et al.'s 1980 study included a sheep feeding trial with a select number of mushroom waste-based feeds, the report was preliminary, and therefore does not include the results of the feeding trial. However, a later paper by the same authors does discuss the results of a lamb metabolism trial using ensiled hay, corn and mushroom waste feed (Wilson, 1983). This study tested three diets containing 10% hay, 15% corn, and 75% mushroom waste, the latter 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 it should be noted that the low nutritional value of *A. bisporus* waste could be due to the low nutritional value of the initial substrate, which is typically composed primarily of horse manure (Wilson, 1983). Other mushroom species which are grown on more nutritious substrates may produce SMS of higher nutritive value.

Pleurotus sp., commonly known as oyster mushrooms, are the second most cultivated mushroom worldwide (Islam et al., 2017), accounting for 27% of global mushroom production (Rinker, 2017). Oyster mushrooms are well known because they are easy to grow, highly nutritious, and can be grown on a wide variety of agricultural wastes (Mohd Hanafi et al., 2018). *Pleurotus* sp. are high in protein (15-35%) 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 (Stamets, 2000). Not only can oyster mushrooms be grown on waste materials, allowing for incorporation into circular nutrient cycles, but these substrates may actually benefit mushroom production. The high carbon and nitrogen content in agricultural wastes improves the nutritional quality and protein content of mushroom fruiting bodies grown on these materials, compared to more commonly used commercial substrates such as sawdust (Mohd Hanafi et al., 2018).

A 1998 feeding trial by Adamović et al. studied chemical changes brought on by *P. ostreatus* grown on wheat straw, and the use of the resultant SMS as a cattle feed in a feeding trial. The results of the laboratory analysis showed that cell-wall components of the straw, especialy lignin and cellulose, decreased during incubation due to degradation by *P. ostreatus* enzymes, corresponding with an increase in CP and free sugar content. Processing by *P. ostreatus* therefore increased the protein content and digestibility of the substrates, a promising result which is unfortunately mitigated by the results of the feeding trial. Despite a theoretical imporvement in feed quality, the average daily gains were smaller in both groups consuming SMS (at 10% and 17% respectively) compared to a control group eating their regular feed. This result can be attributed to low palatibility of the SMS feed - during the trial, the cattle rejected SMS unless it was mixed with silage, and refused to consume anything more than 17% SMS (lowered from the original trial goal of 20%). Although the daily gains were less in both SMS feed groups compared to regular diets, the group consuming 10% SMS had only 10g less gain than the control group, compared to 60g less for the group consuming 17% SMS. It is difficult to determine how much of this reduciton is due to reduced feed intake, and how much is due to the quality of the feed itself. Therefore, if a solution could be found that would increase the willigness of cows to eat the SMS feed, *P. ostreatus* SMS could be a valuable feed additive to increased protein intake for livestock.

While the use of agricultural wastes 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 (Glück-Thaler, 2012). One issue found in this research was the increased risk of contamination when using SCG substrates; they found that when contamination occurred (in the form of green mold) it was on the top layer, and suggested the addition of a grain spawn layer on top of the substrate surface to give the mycelium more of an advantage. 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 and (when combined at the proper ratio) they provide a free, high quality substrate on which to grow oyster mushrooms. Despite the promising implications of being able to grow a high-quality food source on urban solid waste, very 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 (van Hullebusch et al., 2021), this research gap should be rectified. This paper will contribute by focusing on the valorization of urban waste by fungal bioconversion to both human and cattle food.

When using carboard and SCG as a fruiting substrate, the SCG is typically added at lower quantities as a supplement to increase protein content, which helps to increase yields (Stamets, 2000). However, as mentioned previously, SCG addition also increases the risk of contamination, so a balance must be found between nutritional quality and contamination risk. In a previous study conducted by the researchers, three different ratios of cardboard to SCG were tested to determine which would be most effective at producing high quantities of mushrooms without becoming contaminated. In this experiment, grey oyster mushrooms (*Pleurotus ostreatus var. columbinus*) were grown on five substrates with coffee contents of 0%, 25%, 50%, 75% and 100%, with cardboard composing the rest of the substrate. Both treatments with a coffee content higher than 50% effectively failed, producing little to no fruit. The treatment with no coffee also performed poorly, but still produced some fruit. The treatments with 25% and 50% coffee were the best performing, with approximately equivalent fruit production, almost three times the amount of the 0% coffee treatment.

Based on the results of this previous research, three substrate treatments were devised, all with lower than 50% coffee content: 0%, 20% and 40% coffee. The main goal of this study was to determine if SMS from oyster mushrooms grown on carboard and SCG could be used as cattle feed, so SMS nutritional content will be the measured value of interest. To determine feed suitability, the question being addressed is what substrate ratio of cardboard to coffee would result in the most desirable nutritional profile. An additional question of interest is if pre- or post-fruiting samples would be more suitable for feed. Due to the lignocellulosic breakdown of mushroom digestion, it is hypothesized that post-fruiting substrates would be most desirable as feeds due to reduced fiber and increased protein content, however because loss of organic matter is highest during fruiting the nutritional value (and feed quality) of the SMS could be theoretically decreased post-fruiting (Zhang et al., 1995). It is necessary to test the change in nutrition as a result of fruiting due to these competing assumptions.

In order to compare the nutritional content of SMS feed to traditional feeds, the samples will be tested for crude protein, moisture, NDF and mineral content. The NDF content is a measure of cell wall content and therefore used to determine fiber content and digestibility (Reed, 2004), as well as being a predictor of voluntary intake of feed (Thunes, 2019). Therefore, this experiment will measure the nutritional value of the mushroom substrates both before and after mushroom harvest in order to inform decisions regarding which stage to pull substrates for feed, and what substrate ratio to use for the most desirable nutritional profile.

MATERIALS AND METHODOLOGY

Materials

1. Substrates

All substrates used were diverted from the waste streams of local Montreal businesses.

Cardboard was collected from a recycling bin behind a grocery store. During collection, discarded boxes used to receive shipments were examined, and only cardboard that was clean and without visible glue or ink was selected.

Coffee was collected from Café Neve with help from employees, who placed the SCG's in a closed container after brewing for the span of a business day. This container was then collected at the end of the business day. Almost 2 kg of grounds were collected, and the employees stated that this was less than a typical day.

2. Mushroom Spawn

Mushroom spawn was purchased in 1kg quantity from Mycoboutique (Montreal, QC), a mushroom supply store in Montreal. The strain used was *Pleurotus citrinopileatus* (Yellow Oyster, or Golden Oyster), as it was the only one available at the time of purchase. While less popular with mushroom growers than other strains such as *Pleurotus ostreatus,* this species has comparable growing requirements and nutritional value to more well known oyster strains, and will be used interchangeably.

3. Fruiting Chamber

A shotgun fruiting chamber (SGFC) was constructed following instructions on the FreshCap Growing Blog (Shields). 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 (SpitballJedi, 2014).

4. Other Notable Materials

The mushrooms were grown in #4T polypropylene bags, which had a 0.2 micron filter patch to allow air flow while preventing contaminants from entering during colonization (Mycoboutique, Montreal, QC).

Sterilization of tools and surfaces was done with 70% isopropyl alcohol.

A generic kitchen scale with ±1g accuracy was used to weigh the substrates and spawn.

Methodology

1. Preparation

To avoid contamination, all substrates were pasteurized before inoculation.

Cardboard was cleaned using hot water pasteurization (EZMushroom, 2021). The boxes were cut into large pieces, placed in a large sturdy plastic storage container and soaked in boiling water for two hours. After soaking, all excess water was shaken off the pieces, which were then stacked and covered.

After being pasteurized in the brewing process, the SCG's were placed in a sealed container and were then used within 24 hours of collection. Therefore, no further sterilization was performed on the coffee.

Due to lack of a sterile work area (such as a laminar flow hood), an alternative technique was used to further reduce contamination risk during inoculation. This method was compiled according to various tips from users on a mushroom forum post which has since been deleted, so unfortunately no reference is available. After the substrates were prepared, and all necessary materials collected, the inoculation room was sealed off by closing the door and placing towels in the cracks. Then, the substrates and materials were covered with a drop cloth. Once all work surfaces were covered, water was misted throughout the room, focusing on the ceiling and corners. After misting, the doors remained closed until all treatments were prepared, ensuring that any air-borne contaminants are precipitated to the ground, reducing contamination risk. Fifteen minutes after misting, the drop cloth was removed, and all surfaces (including the scale and bowls for weighing substrates) were sprayed down with 70% isopropyl alcohol. After this had completely evaporated, inoculation began.

2. Inoculation Procedure

Three different treatments were prepared at different cardboard to coffee ratios, all with a 20% spawn rate and the same total substrate weight. The substrate ratios for each of the three treatments is displayed in table 1 below. Three bags were prepared for each treatment, for a total of nine inoculated bags.

Table 1. Summary of substrate compositions for each treatment.

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

For each trial, the cardboard was torn into squares that were approximately the size of the bag, then layered in the grow bags with coffee and/or spawn, with cardboard layers being 1-3 pieces of cardboard thick. The bags were then sealed with zip ties above the filter patch to allow air flow during colonization, placed in a dark room out of direct light (but not completely dark), and left to colonize. The bags were fully colonized after 21 days, after which fruiting began.

3. Fruiting Procedure

The bags were cut under the zip tie, to allow access to the substrate for sampling, which is detailed in the section below. After sampling, the open end of the bag was folded over and taped firmly to the back of the bag, to ensure no air pocket existed at the top of the bag, as this would encourage fruiting in an undesirable area. About halfway down the front of the bag, a 1 in. incision was then made, from where the mushrooms would fruit.

The bags were then placed in the SGFC, on top of the moistened perlite. The SGFC was misted 3-6 times a day for the duration of fruiting. The fruiting time varied greatly for each bag; the first fruits (from bag 3C) were harvested 12 days after the bags were placed in the SGFC, compared to 38 days for the last fruits (from bag 1A) (on September 16).

4. Harvesting

Once the fruits were ready for harvesting, indicated by the flattening of the mushroom caps, they were harvested by incision with a knife at the base of the mushroom, where they exited the bag. After harvesting, the fruits were weighed and then refrigerated for consumption.

5. Sampling

Pre-fruiting sampling was very conservative, as too much disturbance of the substrate could have impacted the health of the mycelium, leading to higher risk of contamination and issues during fruiting. Six samples were taken from various locations in the bags, for a total weight of approximately 10 g per bag. After samples were taken, they were placed in a Ziploc bag, labelled, and placed in the freezer.

After all bags had fruited, frozen bags were thawed, and then samples were taken. Each bag was cut open, and the contents ripped apart as much as possible and mixed up in a large bowl. Then 200 g samples were taken in small, random increments from the bowl, then placed into a Ziploc bag and labelled.

6. Testing

After all samples had been retrieved, they were sent to Agrianalyse (Sherbrooke, Quebec). The samples were all tested using wet chemistry techniques.

7. Statistical Analysis

After the testing results were obtained, they were imported to Excel and then checked for normality using the Shapiro-Wilke test.

One-way ANOVA tests were performed on normal data sets to determine if the difference between the means of the three treatment groups was statistically significant (where statistical significance is indicated by a p-value of less than 0.05).

For non-normal data sets, and where necessary for normal data sets, an independent two sample t-test was performed to determine if there was a statistically significant difference between two treatment means.

RESULTS

The first round of sampling was done after the incubation period, when the bags had been fully colonized by mycelium (28 days after inoculation) but before fruiting had initiated. These pre-fruiting samples would have undergone some lignocellulosic breakdown through mycelium digestion, but not to the extent of post-fruiting samples, so these samples were taken as a baseline to compare with post-fruiting nutritional values. The second round of samples were taken after one flush of mushrooms had been harvested, with testing results shown in table 2.

Table 2. Complete post-fruiting values for each treatment.

Feed Parameter	Treatment 1	Treatment 2	Treatment 3
Protein % (Nx6.25)	5.353 ± 3.969	5.200 ± 0.0346	6.597 ± 0.274
NDF %	78.53 ± 4.06	73.70 ± 3.76	71.67 ± 2.91
Calcium %	1.45 ± 0.54	1.46 ± 0.39	1.24 ± 0.44
Phosphorous %	0.055 ± 0.007	0.070 ± 0.014	0.075 ± 0.007
Potassium %	0.087 ± .006	0.177 ± .006	0.237 ± .006
Magnesium %	0.083 ± .012	0.103 ± .015	0.100 ± .017
Sodium %	0.060 ± 0	0.050 ± .010	0.040 ± 0

It was hypothesized that these post-fruiting samples would have experienced further lignocellulosic breakdown compared to pre-fruiting samples, corresponding to a decrease in fiber (NDF) content. In order to test this hypothesis, the change in NDF content was calculated from the pre- and post-fruiting values, and a one-way ANOVA test, summarized in table 4, was performed to compare the change in NDF content for each treatment. No significant difference was found for change in NDF between treatments, however descriptive statistics, shown in table 3, did reveal a mean decrease in NDF content for treatments 2 and 3. All treatments had a relatively high standard deviation. The change in NDF content for all three treatments are plotted below in figure 1.

Table 3. Change in Fiber Content: Descriptive Statistics.

Treatment	N	Mean	Std. deviation	Minimum	Maximum
1 (0% Coffee)	3	0.533	4.055	-3.4	4.70
2 (20% Coffee)	3	-3.900	3.764	-8.0	-0.60
3 (40% Coffee)	3	-4.233	2.914	-7.50	-1.90

Table 4. Change in Fiber Content: ANOVA results.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	42.487	2	21.243	1.630	0.272
Within Groups	78.213	6	13.036		
Total	120.700	8			

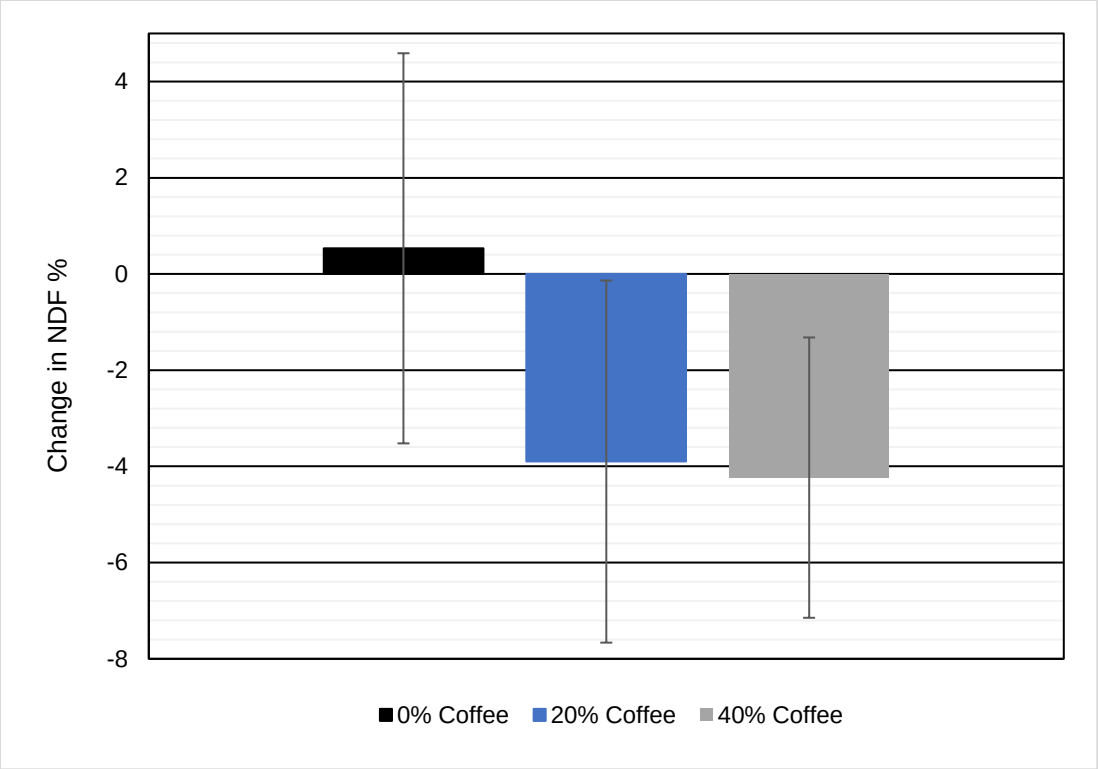

Figure 1. Change in fiber content in SMS as a result of mushroom digestion.

In order to facilitate comparison to typical cattle feeds, the final NDF content was also analysed using both a one-way ANOVA (table 6) and two-sample t-tests between samples (table 7). Due to high variance in these samples (table 5), statistically significant results were not obtained using either test, however the plot of final NDF content (fig. 2) shows a slight reduction in NDF (which is not statistically significant) with each treatment as cardboard content in the substrate lowered.

Table 5. Final Fiber Content: Descriptive Statistics.

Treatment	N	Mean	Std. deviation	Minimum	Maximum
1 (0% Coffee)	3	78.533	4.055	74.60	82.70
2 (20% Coffee)	3	73.700	3.764	69.60	77.00
3 (40% Coffee)	3	71.667	2.914	68.40	74.00

Table 6. Final Fiber Content: ANOVA results.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	74.647	2	37.323	2.863	0.134
Within Groups	78.213	6	13.036		
Total	152.860	8			

Table 7. Final Fiber Content: Two-sample T-test results for equality of means.

Treatments	Assumption	t	df	Sig. (2-tailed)	Mean difference	Std. error difference
1 and 2	Equal Variances	1.513	4	0.205	4.833	0.291
	Unequal Variances	1.513	2	0.205	4.833	0.291
1 and 3	Equal Variances	2.382	4	0.076	6.867	1.141
	Unequal Variances	2.382	2	0.076	6.867	1.141
2 and 3	Equal Variances	0.740	4	0.500	2.033	0.850
	Unequal Variances	0.740	2	0.500	2.033	0.850

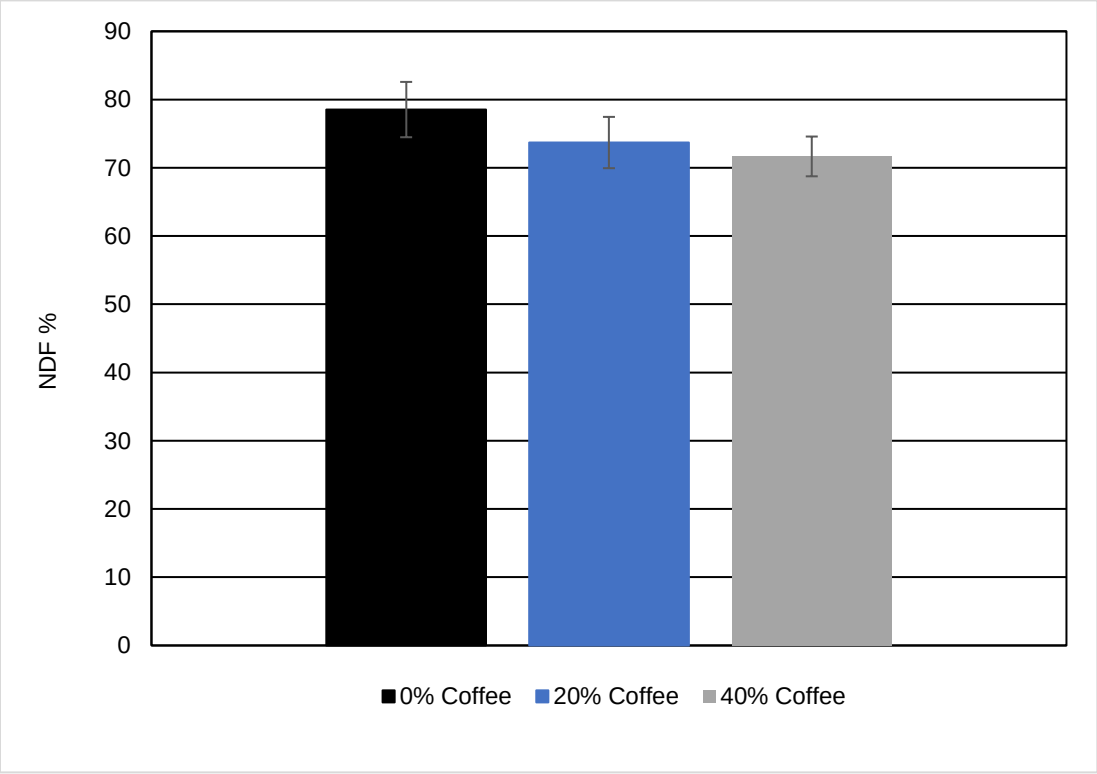

Figure 2. Final (post-fruiting) fiber content of SMS.

Following the results of previous studies, it was hypothesized that protein content would increase as a result of mushroom digestion. However, this increase could have been mitigated due to the removal of organic matter in the form of harvested mushrooms, which would reduce the overall protein content in the SMS. Using the pre- and post- fruiting protein values, the change in protein as a result of mushroom digestion was plotted, and as hypothesized all treatments had an increase in mean protein content (fig. 3 and table 8). However, the one-way ANOVA test, the results of which are shown in table 9, did not find statistical significance between the means of the groups, most likely due to high variation in treatment 1, and a lack of normality in treatment 2. A two-sample independent t-Test was also performed to determine if there was a significant difference

within the group means, finding that treatments 2 and 3 had significant differences between their mean change in protein content, with treatment 3 having a larger change (table 10).

Table 8. Change in Protein Content: Descriptive Statistics.

Treatment	N	Mean*	Std. deviation	Minimum	Maximum
1 (0% Coffee)	3	1.973	3.969	-0.730	6.530
2 (20% Coffee)	3	0.640	0.035	0.620	0.680
3 (40% Coffee)	3	1.947	0.274	1.750	2.260

Table 9. Change in Protein Content: ANOVA results.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.486	2.000	1.743	0.330	0.731
Within Groups	31.659	6.000	5.276		
Total	35.145	8			

Table 10. Change in Protein Content: Two-sample T-test results for equality of means.

Treatments	Assumption	t	df	Sig. (2-tailed)	Mean difference	Std. error difference
1 and 2	Equal Variances	0.582	4	0.592	1.333	3.934
	Unequal Variances	0.582	2	0.620	1.333	3.934
1 and 3	Equal Variances	0.012	4	0.991	0.027	3.695
	Unequal Variances	0.012	2	0.992	0.027	3.695
2 and 3	Equal Variances	-8.186	4	0.001*	-1.307	-0.240
	Unequal Variances	-8.186	2	0.015*	-1.307	-0.240

***Statistically significant mean difference**

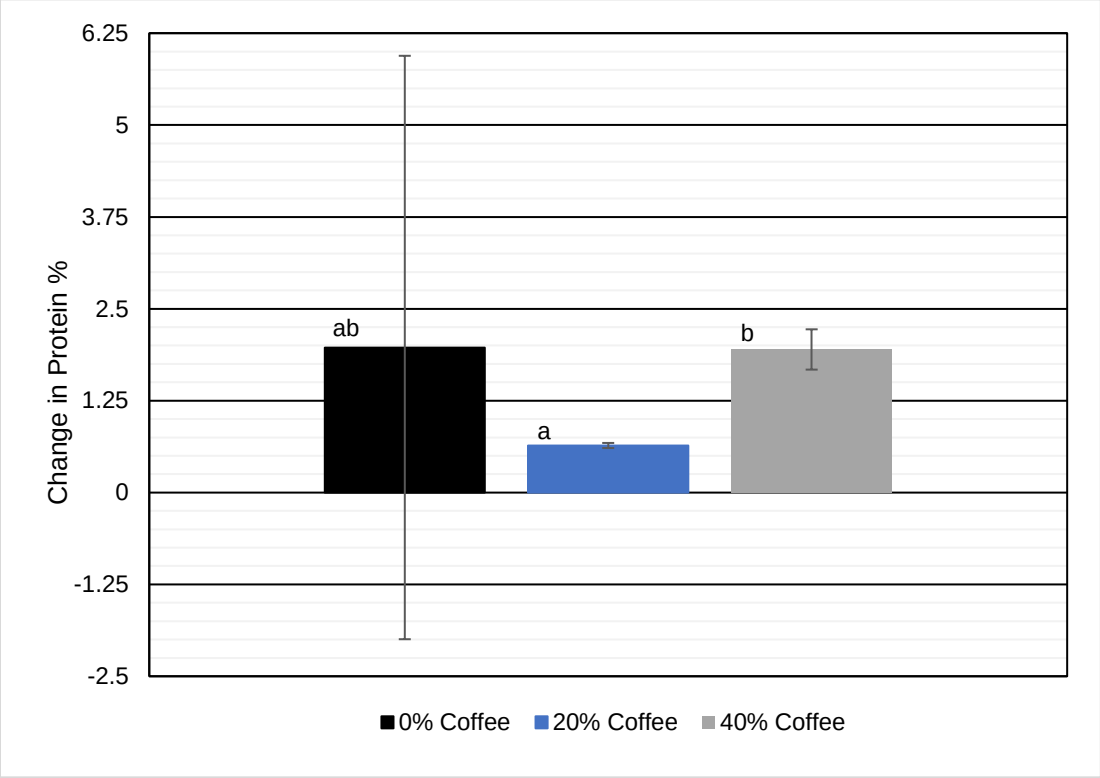

Figure 3. Change in protein content in SMS as a result of mushroom digestion.

To answer the question of which substrate yields the most desirable nutritional values for feed, final protein content was analyzed for each treatment. A one-way ANOVA test performed on final protein content showed no significant difference between group means (table 12), however once again high variation in treatment 1 is likely a factor (table 11). A two-sample t-test did reveal a significant difference between the means of treatments 2 and 3, with treatment 3 having a higher protein content (table 13). The results of this analysis are plotted below in figure 4.

Table 11. Final Protein Content: Descriptive Statistics.

Treatment	N	Mean	Std. deviation	Minimum	Maximum
1 (0% Coffee)	3	5.353	3.969	2.65	9.91
2 (20% Coffee)	3	5.200	0.0346	5.18	5.24
3 (40% Coffee)	3	6.596	0.274	6.4	6.91

Table 12. Final Protein Content: ANOVA results.

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	3.520	2	1.760	0.334	0.72
Within Groups	31.659	6	5.277		
Total	35.179	8			

Table 13. Final Protein Content: Two-sample T-test results for equality of means.

Treatments	Assumption	t	df	Sig. (2-tailed)	Mean difference	Std. deviation difference
1 and 2	Equal Variances	0.067	4	0.950	0.153	3.934
	Unequal Variances	0.067	2	0.953	0.153	3.934
1 and 3	Equal Variances	-0.541	4	0.617	-1.243	3.695
	Unequal Variances	-0.541	2	0.643	-1.243	3.695
2 and 3	Equal Variances	-8.750	4	0.001*	-1.397	-0.240
	Unequal Variances	-8.750	2	0.013*	-1.397	-0.240

*****Statistically significant mean difference

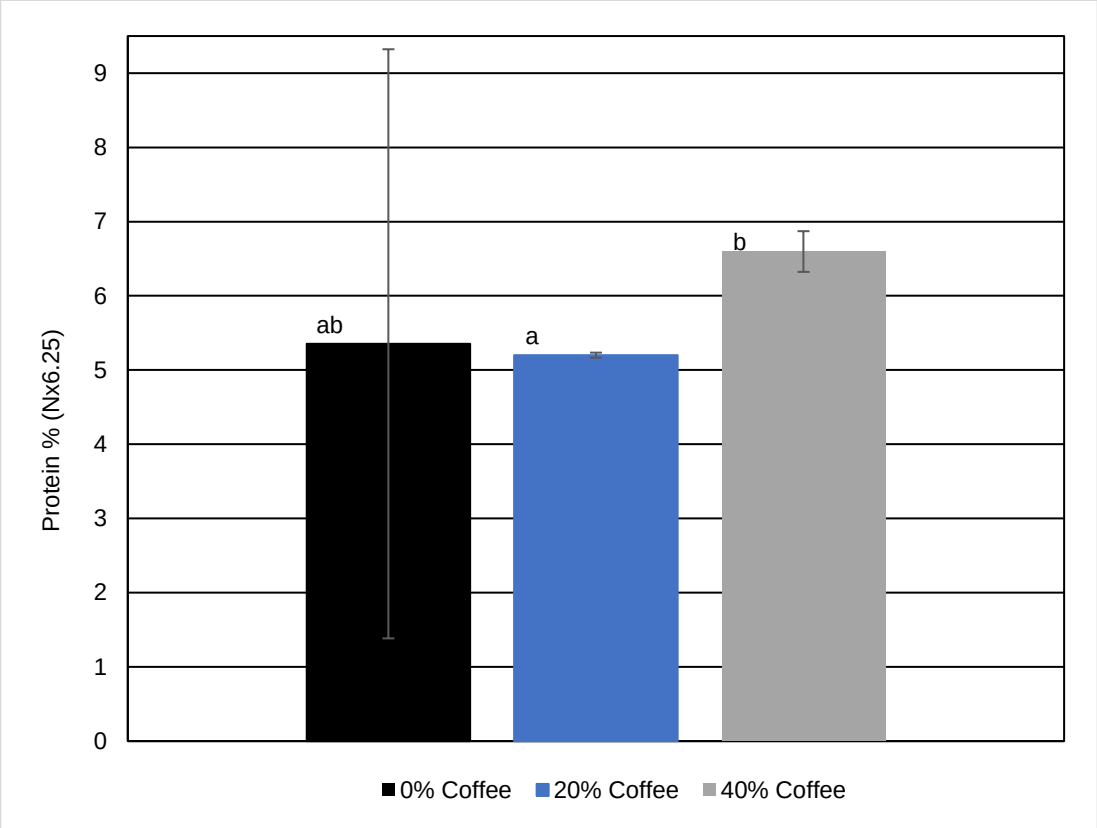

Figure 4. Final (post-fruiting) protein content of SMS.

DISCUSSION

The main question being addressed in this study was which substrate would result in the best SMS for use as feed, and if this would occur before or after fruiting. All treatments experienced statistically significant increases in protein, and a trend of decreased NDF content (which lacked statistical significance), supporting the hypothesis of mushroom degradation leading to an increase in feed digestibility and quality, a desirable outcome for this study. Therefore, substrates can be pulled post-fruiting for us as SMS feed, which gives the added advantage of oyster mushroom harvest. One issue that was highlighted in the literature review was the high variability of SMS nutritional content, and this issue arose in the study, with treatment 1 having the highest variation. This outcome, combined with high NDF and lower protein in treatment 1, allows us to disqualify it as a potential feed.

Treatment 3 had the highest change in protein (1.947%) and final protein content (6.596%), 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 final results for these treatments are compared to typical dairy cow diets below in table

14. As predicted, both treatments had much higher protein contents than these typical diets, with over three times the protein 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 unfortunately detracts from the benefits of high protein content due to the inverse correlation between digestibility, voluntary intake and NDF content.

Table 14. Typical diet formulations for early, mid and late lactation cows, compared 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
Forage DM as proportion of total DM	0.3	0.5	0.7		
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 ± 3.9	12.4 ± 4.4
Phosphorus (g kg^{-1} DM)	4.5	3.5	3.0	0.70 ± 0.14	0.75 ± 0.07
Magnesium (g kg^{-1} DM)	1.8	1.5	1.5	1.03 ± 0.15	1.00 ± 0.17
Sodium (g kg^{-1} DM)	1.8	1.5	1.5	0.50 ± 0.10	0.40 ± 0

All diet data from (Phillips, 2004) unless noted

*(Erickson and Kalscheur, 2020)

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 (Garnsworthy, 2004). 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 (Goodwill, 2004). 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 because they commonly use sugarcane molasses as binding agents (Phillips, 2004). Additional advantages of pelleting include enhancement with additives for a number of reasons, from nutritional value to medical benefits to increased palatability (Goodwill, 2004; Rouchouse, 2020). On-site pelleting could make transportation of SMS easier and more economical, however the overhead costs of pelleting must be considered.

CONCLUSION AND RECOMMENDATIONS

The main goal of this study was to determine if SMS from golden oyster mushrooms (*Pleurotus citrinopileatus)* grown on carboard and SCG could be used as cattle feed. This was done by determining what ratio of cardboard to coffee would result in the most desirable nutritional profile, and also if pre- or post-fruiting samples were more suitable for feed. Treatments 2 and 3, containing 20% and 40% coffee respectively, were found to be suitable for further study due to their high protein and calcium content post-fruiting. Treatment 1 was dismissed due to lower protein content, and extreme variability. Despite the added nutritional value resulting from mushroom processing of the substrates, there are issues that must be addressed in order to translate these findings to a practical, applicable animal feed solution. The two most pressing issues found in this paper were palatability, high NDF content and high variability in nutritional content. 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

In addition to nutritional advantages offered by SMS feeds, there are also potential economic benefits - the high input cost associated with 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.

Recommendations for Future Research

The limited scope of this project did not allow for the investigation of many factors. Further research must be done to assess the practical applications of the results presented here. A full feeding trial must 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. (1998) may be of use in designing these trials.

Additionally, it would be valuable to conduct an economic analysis to further assess the practicality of implementing these recommendations, and to encourage farmers and mushroom producers to pursue these changes, which can be an economic risk. It may also be beneficial to determine if other lignocellulosic wastes could be used in place of cardboard to reduce the NDF content of the SMS. One other possibility is the use of SMS for mealworm feed, rather than cattle feed, as insect nutrition is gaining awareness as a sustainable protein source.

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