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Potentials of Pleurotus: Reimagining the Relationship Between Cattle and Brewer's Spent Grain

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Potentials of *Pleurotus*: Reimagining the Relationship Between Cattle and Brewer's Spent Grain

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by
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This work is dedicated to Riška

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I would like to thank my friends for sharing my excitement about this project and making me smile all the way through. Thank you to my parents, who have taught me the value of hard work.

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Table of Contents

Abstract

Brewer's spent grains (BSGs), a byproduct from beer production, are generated in excess globally. Most often, they are sold or given to proximate cattle farmers for use as feed. However, spent grain can also be used as a medium for fungal cultivation. Given that certain fungal species have the capability to degrade lignin and produce protein, the cultivation of fungi on spent grains may serve to enhance the nutritional profile of the grains for their use as cattle feed. This project is an effort to determine the compatibility of fungal cultivation with BSGs in order to both improve upon cattle diet and potentially provide the farmer with a secondary income stream from fungal cultivation. Brewer's spent grains were obtained from two sources: Lasting Joy Brewery in Tivoli, NY, and from a homebrew process using a beer homebrewing kit. The experiment occurred outside of a lab setting using commonly utilized household materials in order to determine the feasibility of this project in real-world applications. Spent grains from each source were sterilized, and then inoculated with *Pleurotus eryngii*, or directly inoculated in order to determine whether the beer brewing process provides sufficient pasteurization to prevent contamination from other organisms. The spent grains were sent to a laboratory for nutritional analysis both before and after they were inoculated with fungi. Successful mycelial development was exhibited in one sample. Due to the small sample size, statistical tests were unable to be performed on the results of the nutritional analysis. The effect of fungal growth on brewer's spent grain in terms of nutrition is unclear, however, fungal cultivation on spent grains using low-cost materials was possible and shows promise for future permutations of this project.

> *The waste in our existing food production system comes from pulling food out of the loop between soil and eaters, commodifying it, bashing it around to its nutritional detriment, and selling it back to consumers who have already paid for it once with subsidies and will fork over at the cash register and then pay again at the doctor's office.*
> –Joann S. Grohman, *Keeping a Family Cow,* 2013

1. Introduction

1.1 Introduction

Farmer Alejandro Carillo, of Rancho Las Damas in the Chihuahuan desert, has dedicated his life to restoring wildlife and regenerating his dried lands with the use of his cattle herd. He sees the persistent drought conditions and lack of vegetation as the product of mismanaged conventional grazing practices and has developed a rotational grazing system in which his cows are passed through fenced areas on a schedule, allowing for their fertile manure to enhance topsoil and encourage latent seeds to germinate. As he tours the cameramen around his ranch in *Sacred Cow* (2020), he comes across a cow pie lying on the grass in front of him. Reaching down to examine it, he holds it up to his nose, appreciating its distinctly earthy odor as if he has never smelled it before. And then he notices a mushroom fruiting right off of the cow pie, remarking, "Pretty amazing to have a mushroom here, even in the Chihuahuan desert" (*Sacred Cow*, 2020).

The spirit of this image– of a cow pie fostering the growth of a mushroom, even in the driest desert conditions– is that of a generative partnership between two grossly misunderstood organisms. Mismanagement of cattle husbandry in the United States and its linkages to climate change via methane and nitrous oxide emission have led to the widespread demonization of cattle farming in general, no matter how it is performed. Mushrooms share a similar fate– their long-standing Western association with death and decay has contributed to severe research gaps and funding scarcity in the field of mycology (Kaishian & Djoulakian, 2020). The gloomy

associations fostered towards each of these organisms thus wholly cease any fruitful and natural relationships that may be had between them, and removes their agency as organisms outside of human culture. If we overcome these beliefs and lean into the chaotic entanglements that comprise the natural world, we can defy the prevailing sterile monoculture cropping complex that threatens to compromise the Earth and its living beings forever.

1.2 Fungi Exemplify Circular Food Systems

Circular food systems honor this interconnectedness between organisms. A circular food system, rather than a linear model, centralizes the principles of sharing and re-use to support tighter networks of food production where people are closer to the source of their food (ICLEI - Local Governments for Sustainability, 2021). A principal example of a circular food system comes from a sorghum beer brewery in Namibia. The wastewater from Tunweni brewery is mostly comprised of unsold beer and water used to clean the brewing facilities. This fluid waste, along with wastewater from nearby office buildings and animal manure, is recycled with the help of bioprocessing. This is a method by which living organisms are able to significantly transform a material to create a ‘value-added’ substance by means of their natural functioning (Cossar, 2011). The wastewater is filtered through a succession of integrated biological systems: fungi and earthworm beds, a pig sty, anaerobic and aerobic digesters, and algae ponds. This wastewater is eventually incorporated into large fish ponds, where the resulting nutrient-dense water is able to sustain the development of fish without the addition of costly fish feed supplements (Okeyo, 2000). This nutrient-rich water along with fish waste is able to encourage a robust food chain composed of phytoplankton, zooplankton, and invertebrates (Okeyo, 2000). Thus, there is little left to waste at this facility and many opportunities for growth and regeneration.

Fungi exemplify the core tenants of a circular food system by their very nature. They exhibit unique abilities to flourish on a diverse set of unconventional substrates, including coffee grounds and cardboard (van Wyk, 2021), chaff remaining from threshed millet and sorghum (Ryden et al., 2017), and spent sorghum grains from beer brewing and chopped grass (Okeyo, 2000). Fungi are generally known to be difficult to cultivate on industrial scales, and thus subvert notions of capitalistic production and favor small-scale local usage or foraging practices. Furthermore, the cultivation of fungi on intimate scales will be critical for farmers adjusting to differing conditions due to climate change inputs (Tesfaw et al., 2015), like drought, which adversely affects the yields of crops that are heavily relied on as food and income sources. Certain fungal genera, like oyster mushrooms, require few environmental controls, are generally not susceptible to degradation from pests or pathogens (Sánchez, 2010) and are conducive to growth in conditions that do not mimic high-cost laboratories (Tesfaw et al., 2015). Besides being tasty and important to many different cultures across the world, oyster mushrooms are a good source of protein, carbohydrates, dietary fiber, amino acids, lipids, and an assortment of water-soluble vitamins and minerals like folic acid, which is an essential vitamin that cannot be synthesized by the human body and thus requires dietary supplementation (Raman, 2021). Depending on the type of substrate, which refers to the material utilized as the sort of ‘soil’ on which the fungi develop and uptake nutrients from, cultivated oyster mushrooms may have excellent biological efficiency levels (Sánchez, 2010; Wang et al., 2001). This means that the percentage of the substrate (often composed of substances like grain, sawdust, or straw) that is converted into biomass, or edible fruiting bodies, is high.

It has become an increasingly favorable standpoint to look towards fungi as the solution to the myriad environmental and economic problems caused by reliance on the extractive,

polluting modes of industrialism. Filamentous fungi are celebrated for their ability to serve as meat replacements, packaging material, insulation, and leather alternatives (Meyer et. al., 2020). This prevailing understanding of fungi as 'saviors' requires further examination so as not to slip into the extractive tendencies that engendered resource scarcity and environmental degradation in the first place. In order to incorporate fungi into our systems as remediators, it is important to honor them first as an organism of their own, and celebrate their countless other roles in natural systems. Along with relying on fungi to alleviate the issues presented to people and the environment, we must also effectively learn to protect fungal habitats, which seemingly exist outside of the realm of productivity and profit. Some fungi are recognized as ecologically cryptic, meaning that they subsist in an environment yet do not make themselves known with any distinguishable features besides "microscopic hyphae and mycelia" (Hawksworth & Lücking, 2017). Others are considered semi-cryptic, meaning distinguishing one species from another is indubitably difficult, especially if a species is assumed to be a part of another recognizable species or group, thus becoming repeatedly concealed under that false categorization (Hawksworth & Lücking, 2017). The critical underfunding of mycology as an area of research in tandem with these variables has contributed to the largely misunderstood role of fungi in ecosystems, as well as their sheer diversity as a kingdom.

A result of this is that a nearly unfathomable number of fungal species remain completely undescribed. The most cited paper on the subject reports that there are 2.2 to 3.8 million undescribed fungal species (Hawksworth & Lücking, 2017), while other papers announce that this number is more like 6 million (Taylor et al., 2014), or even up to 11.7 to 13.2 million species (Hyde, 2022). The most recent estimate of described fungal species is 150,000 (Hyde, 2022). The numeric value associated with fungal diversity is difficult to agree upon, likely due to the

confounding factors of fungal inscrutability and mycology's undervaluation as a field of study. This uncertainty makes gauging the extinction or endangerment status of these unknown species difficult to report, which dually contributes to the lack of protective standing afforded to these organisms and the lack of funding available to mycologists that ardently study these organisms to have the means to do so (Kaishian & Djoulakian, 2020, p.23). It is crucial, then, to privilege fungi as organisms for all that they are; to keep investigating their role as effective remediators, yes, but also to care for and probe into fungal networks even if the 'return' is nowhere in sight.

1.3 This Senior Project

With this framework in mind, fungi offer opportunities to restore and enhance wasteful and ineffective food systems, which are in dire need of critical revision. They are able to do so by working together with other organisms, mirroring what nature intended, and potentially alleviating the financial burdens of marginalized populations as a result. This project calls upon fungal expertise to mitigate the challenges between two entangled industries: beer brewing and cattle husbandry.

It is a common practice around the world for beer breweries to sell or give their spent grain from beer production to local farmers for use as cattle feed (Bolwig et al., 2019; Mussatto et al., 2006). The usage of a viable waste product to supplement the feed of cattle is an incredibly important step towards lessening both the impact of the beef industry and the brewing industry on the environment. Cattle have the unique quality of being able to digest all fibers, besides lignin, in their specialized ruminant digestive systems. For this reason, cattle are able to consume a wide variety of food sources otherwise indigestible to other organisms, turning this fibrous fuel into indispensable food products like beef or milk. Brewer's spent grains (BSGs), like all grain

fed to cattle, are lignocellulosic– meaning that the cell walls of the grain are partially encased in stalwart lignin– and render the rumen unable to effectively gain access to the cellulose and hemicellulose (both important carbohydrates) in the spent grain (van Kuijk et al., 2015a). BSGs could use some improvement when it comes to meeting the dietary needs of cattle, so that farmers are able to rely on it as a food source and circumvent the usage of fresh grain and oilseeds in their feed.

Fungi, specifically white rot fungi or wood-decaying fungi, are able to break down the lignin present in the material that they have populated. *Pleurotus* spp. not only break down lignin using extracellular digestive enzymes, but selectively degrade the lignin and leave other nutrients intact (Abdel-Hamid, 2013). This, among other qualities, makes *Pleurotus* spp. compelling candidates for enriching the nutritional value of spent grain when it becomes incorporated into cattle diets. Before the fungal fruiting stage of certain fungi (when fungi form the recognizable fruiting bodies colloquially known as mushrooms), fungi create dense networks of hyphae called mycelium. This mycelium breaks down the medium it is growing on using secreted enzymes, populating it with white, fuzzy veins. In this stage, the fungi will have broken down most of the lignin in the substrate, or growing medium, as energy for its development, while leaving the valuable cellulose and hemicellulose undigested (van Kuijk et al., 2015a). This is the phase at which cattle might benefit the most from ingesting the myceliated spent grain. However, using a small portion of this myceliated grain can also act as grain spawn, a term used in mushroom cultivation to refer to the colonized grain which will serve as an inoculant to a larger chunk of pasteurized or sterilized material. This process is conducive to fruiting– where the fungi can be harvested and utilized as an alternative income stream for farmers or brewers alike.

The aim of this project is to not only assess fungal abilities in delignifying spent grain and fortifying it with protein, but also to determine whether the fungi can be successfully cultivated utilizing spent grain as a substrate. Could this process be a practical, low-cost and low-energy endeavor that may positively supplement the diet of cattle while simultaneously providing a fungal food or income source to farmers?

In this project, I will obtain brewer's spent grain from two sources: Lasting Joy Brewery in Tivoli, NY, and from a homebrew beer kit. I will inoculate both sources of the freshly spent grain with liquid fungal culture using two treatments– the first will use a DIY still air box to prevent contamination, and the second treatment, acting as a control, will involve sterilization of the grain using a pressure cooker. This will determine if the spent grains are sufficiently pasteurized from the heat generated in the brewing process to support mycelial growth. These four grain samples will be continually monitored for mycelium growth. If the mycelium is able to successfully establish itself on the spent grains, one of the samples will be added to a bulk substrate, or larger growing material, to prepare them for fruiting. This substrate, chopped straw, will be pasteurized using a low-energy method called cold water lime pasteurization. I will simulate ideal fruiting conditions for the straw combined with grain in hopes of achieving a mushroom harvest.

The spent grains from both sources will be sent to a cattle feed testing lab in Ithaca both before they have been inoculated and at the stage at which they are colonized by mycelium before addition to a bulk substrate. The lab testing will determine the nutritional content of the spent grain before and after it has been inoculated with a fungal culture which will determine whether or not the fungi were significantly successful in lignin degradation and the addition of Crude Protein (CP).

2. Brewer's Spent Grains (BSGs) and *Pleurotus eryngii*

2.1 General Properties of Brewer's Spent Grains

Brewer's spent grains are the most abundant byproduct in the beer production process, composing about 85% of waste that emerges from brewing (Lynch et al., 2016). Beer production begins with a variety of fresh grains, of which barley is the most typical in Western beer brewing. Other popular grain choices vary by region; they include sorghum, wheat, rye, millet, corn, and rice (*American Scientist*, 2019; MacLeod,& Evans, 2016). The fresh barley grains are transformed into malt at a malting facility, where they are steeped, germinated, and kilned (Zeko-Pivač et al., 2022), modifying their physical constitutions to activate enzymes important in the brewing process (MacLeod,& Evans, 2016). Next, the malt is blended with water in the mashing stage. In this step, enzymes partially break down starches and proteins present in the malted grain and are converted into sugars that eventually become alcohol (Zeko-Pivač et al., 2022). The water that emerges from this process is called wort, and it is filtered out from the now-spent grains as it continues onwards to become beer (Zeko-Pivač et al., 2022).

The cell walls of both fresh and spent grains have a high content of tough, fibrous lignin, and thus the material is considered lignocellulosic biomass (van Kuijk, et al., 2015a). Lignin comprises 10-28% of spent grains (Lynch et al., 2016). Another key constituent of BSGs is protein, which makes up anywhere from 20% (Mussatto et al., 2006), to 26-30% (Wen et al., 2019) of spent grains. On top of these building blocks, brewer's spent grains consist of a variety of vitamins, some of which include folic acid, niacin, biotin, and thiamine (Ikram et al., 2017). Minerals that compose spent grains in order of highest concentration include calcium, magnesium, phosphorus, and sodium, among others (Ikram et al., 2017). It is important to note that not all BSGs are exactly alike, both chemically and physically– most obviously because

many varieties of grain can be chosen to produce beer– but also due to a host of differences that occur both in the brewing process and in the process of harvesting the grains chosen for brewing (Zeko-Pivač et al. 2022).

There are many factors owing to the notion that spent grains are a notoriously difficult byproduct to dispose of. One of these factors is abundance; annually, about 39 million tons of spent grain are generated worldwide due to beer production (Lynch et al., 2016). To dispose of BSGs, breweries will often sell or give them away to farmers (Bolwig et al., 2019; Mussatto et al., 2006), which accounts for about 70% of the disposal of spent grains (Mitri et al., 2022). Mitri et al. (2022) report that about 10% of spent grains are converted into biogas via microbial anaerobic digestion, and the remaining 20% is either composted (Against the Grain, n.d.; Plant Chicago, 2017) or sent to landfills (Mitri et al., 2022). Timing is critical to these disposal methods– especially if a brewery has no long-term storage capacity available to hold onto spent grains in anticipation of their removal. Brewer's spent grains have a high moisture content of roughly 70-81% (Thomas & Rahman, 2006; Mussatto et al., 2006; Lynch et al., 2016) and are densely packed with protein and polysaccharides (Lynch et al., 2016). Because of these qualities, spent grain is rendered easily spoiled, and difficult and expensive to transport because of its weight (Mussatto et al., 2006).

The qualities that make spent grain challenging to grapple with can become cost-effective and advantageous given the appropriate treatment, which lies in small-scale and local food systems. The idea that spent grain deteriorates quickly and easily only becomes problematic when taking larger food networks into consideration. Infrastructure, like transportation or storage mechanisms, are forced to shrink to fit the small scale that spent grains demand of its consumers. This idea can be threatening, especially as the number of farms, specifically small farms,

declines (Bolwig et al., 2019; Thomas & Rahman, 2006; Weis, 2013) and local food systems are devalued in the face of global ones. Bolwig and colleagues (2019) note quite negatively that the effective reuse of spent grain "requires the presence of local farmers" (p. 1). It is true that the ephemeral nature of spent grains can be a real barrier to correctly, and sustainably, disposing of them; on the other hand, they are a testament to local farms' integral nature in the creation of food systems that prioritize reincorporating traditional waste products.

2.2 The Role of Scale in Modern Usages of Brewer's Spent Grains

BSGs are at the forefront of many efforts to enrich circular food systems in unconventional ways. Fărcas et al. report that spent grains were successfully incorporated into cookie products edible by humans as a replacement for traditional wheat flour (2021). The addition of spent grains increased the nutritional value of the food item by boosting "protein, fiber, lipids, minerals, total phenols, and antioxidant activity" (Fărcas et al., 2021). Using consumer feedback, Fărcas and their team reported that cookies baked with spent grains also positively affected the sensorial aspects consumers expect of cookies, like smell and texture (Fărcas, 2021). Similarly, two other studies conducted in 2008 and 2022 reported that BSGs can potentially replace some or all of the wheat flour used in bread production (Stojceska & Ainsworth, 2008; Merten et al., 2022). Stojceska and Ainsworth (2008) write that mixing BSGs with the ideal enzyme has the potential to remediate prevalent issues when utilizing BSGs in foodstuffs, like shelf life, texture, and loaf volume.

The level of studies in this area indicates the strong potential that spent grains have to be re-incorporated into food systems at different levels. Currently, many of these methods are being commercially employed within the food system, chiefly by start-up companies looking to

repurpose what was once considered a waste material. Rise products, along with a handful of other bakeries across the country, use spent grains from surrounding breweries that they hand mill in place of flour to make bread and other baked goods. Regrained uses patented methods developed with the USDA in order to recycle spent grains into pizzas, pasta, and baked goods. Take Two is a company that utilizes spent grains to create plant-based milk, but has stopped production due to their parent company and investors pulling their funding.

In addition, spent grains have displayed the ability to be incorporated into what is known as a circular bioeconomy, which upholds similar ideologies to that of a circular food system in that viable biological materials are reused as much as possible. This occurs primarily in the energy sector, where spent grains might be used in the production of biofuels (any fuel that is acquired from biomass). The application of spent grain in this field largely focuses on the introduction of BSGs as a source for the production of bioethanol, which is a biofuel used as an additive in gasoline or bioethanol-powered fireplaces. Spent grains were found to be a potentially viable source of bioethanol by a slew of researchers (White et al. 2008; Xiros et al., 2008). So far, one method of bioethanol production using spent grains has been successfully patented (Birkmire et al., 2010). Bioethanol is considered an important resource for its GHG (greenhouse gas) emission reduction potential as opposed to fossil fuels (Mekonnen et al., 2018). However, current methods of obtaining bioethanol, both in the United States and globally, rely heavily on mono-crops, like corn in the U.S. or sugarcane in Brazil (Mekonnen et al., 2018). If indirect land-use changes are taken into account, like deforestation and conversion of grassland into farmland, the usage of corn-based bioethanol is suggested to practically double GHG emissions related to land-use changes (Mekonnen et al., 2018). Nevertheless, using lignocellulosic materials like spent grains instead of edible crops like corn to produce bioethanol has sufficient

drawbacks as well. Mekonnen and their colleagues report that biofuel production using lignocellulosic mass has a significantly larger water footprint, which refers to both the water used and polluted as a consequence of production, than that of fossil fuels (2018). This presents a problem both for water conservation efforts and water quality. Additionally, a high cost is associated with biorefineries that are capable of processing lignocellulosic biomass, which introduces another hurdle for the integration of BSGs into the bioethanol industry (Mekonnen et al., 2018).

Overall, the difficulty of these efforts to reintegrate spent grain into both food systems and circular bioeconomies is infrastructure. Many studies have confirmed that BSGs are an extremely lucrative material when it comes to the creation of biofuels (White et al., 2008), biodegradable plastic substitutes (Corchado-Lopo et al., 2021), or compounds via bioprocessing, like amino acids, fatty acids, enzymes, and vitamins (Mitri et al., 2022). These studies are encouraged by the fact that obtaining BSGs is low or no cost, and that it is generated in excess globally. Yet the feasibility of the aforementioned solutions are all based upon a critical reevaluation of systems that are not designed to repurpose waste. These studies have yet to materialize into widespread practical applications. However, in the case of reusing spent grains for food products, it is apparent that small-scale operations are able to effectively evade the high cost associated with processing spent grains by using pre-existing methods of food production and sourcing spent grains locally. It is with this sentiment that the viability of this project is assessed. The purpose of this project is to work within this well-established and successful small scale in order to determine whether the effective enrichment of spent grains can be achieved in a low-cost and low-energy manner.

This is not to say that large-scale remediation efforts are not needed, both in this specific case of managing brewery waste or in regard to the manifold issues prevalent in industrial agriculture. It is well established that the emissions driving human-induced climate change can be, in large part, traced back to agriculture. The Intergovernmental Panel on Climate Change asserts that between 21% to 37% of global total emissions stem from agriculture alone (Mbow, C. et al., 2019). Reducing emissions will result from an interplay of solutions geared towards both large and small-scale farming operations, though it is important to note that not all farmers will feel the effects of these emissions to the same degree. For example, subsistence farmers in the global South, who farm either completely or mostly for the purpose of providing sustenance for their families rather than marketing their products, are the population of farmers most vulnerable to climatic variability that is characteristic of climate change (Mercer et al., 2012). This is despite the fact that subsistence farmers contribute the least to agricultural emissions (Mercer et al., 2012). The speed and intensity at which climatic flux is affecting small farmers across the world requires amelioration using methods that strive to improve the economic stability of these farmers while also retaining the sustainable practices that often define them. This project is an attempt to harness both of these capacities in hopes of strengthening the resilience of small farmers– yet the focus on climate adaptation and mitigation for small farmers does not intend to grant industrial agriculture the permission to continue its extractive practices going forward.

2.3 Fungal Cultivation using Spent Grains

BSGs have been successfully employed in mushroom cultivation across a wide variety of conditions, including both high-tech laboratories and other more casual settings. Many of the

qualities of spent grains lend themselves well to mushroom cultivation. Firstly, the moisture content of spent grains (70-81%) is within the range that Pleurotus spp. mycelium can tolerate, which spans between 50 to 75% (Bellettini et al., 2019). With a semi-ideal moisture content, no additional energy, cost, or time need to be expended in order to ensure proper moisture levels of the material– the material comes ready from the moment the beer brewing process is finished. The same goes for sterilization or pasteurization, which have the possibility to be quite energy intensive. Mushroom spawning materials and substrates need to be sufficiently sterile in order to prevent contamination by other opportunistic organisms, such as bacteria or molds. This process typically employs tools that can elevate temperature enough to eliminate lingering organisms, like pressure cookers or autoclaves. During the mashing process, grains are elevated to temperatures of around 78℃ (Mussatto et al., 2006)**.** Sterilization of mushroom spawn materials occurs when a temperature of 121℃ is maintained in a pressurized environment for a length of time appropriate to the size of the material being sterilized, anywhere from 45 to 150 minutes (Shields, 2017b). Pasteurization of materials used for mushroom cultivation, however, occurs at a range of 65℃ to 85℃ (Shields, 2017b), so that the brewing process can be considered a form of pasteurization ideal for fungal cultivation. The same can be said for homebrewing methods, as the grains are steeped in warming water at temperatures starting from 76℃ that will heat up to about 93℃.

2.4 Why *Pleurotus eryngii*

Pleurotus spp. can withstand a variety of environmental conditions and thus lend themselves well to cultivation using low-cost technologies and methods (Raman et al., 2021). The *Pleurotus* genus is also recognized for its adaptability to a wide range of substrates. Of these

tolerated substrates, many include waste products like cardboard and coffee grounds (van Wyk, 2021), as well as a plethora of agricultural wastes (Raman et al., 2021).

In a literature review paper that discusses fungal-treated lignocellulosic biomass as a ruminant feed component, species of fungi cited as most effective are *C. subvermispora* and *P. eryngii*. Both of these species are reported to enhance the in-vitro digestibility of their substrates most significantly. However, it is not clear whether *C. subvermispora* is a fungus that is safe to introduce into rumen diets, as it is not cultivated for human consumption (van Kuijk et al., 2015a). *P. eryngii* displays an elevated ability to be selective in lignin degradation, meaning that cellulose levels are minorly impacted by the burgeoning of the fungus on a substrate. This is especially true before the fungus produces fruiting bodies, which require the usage of hemicellulose and cellulose as a source of energy (van Kuijk et al., 2015a). As the mycelium of white rot fungi like *P. eryngii* proliferates on a substrate, the degradation of lignin and other energy sources are transformed into protein by the fungus (Nayan et al., 2018; Scholtmeijer et al., 2023).

Another benefit of *P. eryngii* is its unique mycotoxin suppression capabilities (Chuang et al., 2020a). Mycotoxins are secondary metabolites that are produced by some fungi. They can have many negative effects on animal health, like decreased growth and weakened immune responses that cause vulnerability to disease and infection (Pier et al., 1980). Mycotoxins may proliferate in animal feed if it is inappropriately stored (Chuang et al., 2020a) , but should not be a cause for concern in this study as *P. eryngii* enzymes that are present in the foodstuff will not create favorable conditions for the release of mycotoxins by other fungi. In addition, *P. eryngii*, otherwise known as the king trumpet mushroom, is a delicious and thus highly marketable fungal

species, making it an opportune organism for a secondary income stream given successful cultivation.

3. Ruminant Diets and Economies

3.1 Introduction to Ruminants

Cows are some of the most recognizable and agreeable mammals included in the ruminant suborder. Their counterparts include sheep, goats, buffalo, giraffes, and deer, all of which are dedicated to peaceful grazing and browsing as a means to construct their herbivorous diet. The alternative definition for ruminant, as given by Oxford Dictionary, is *a contemplative person; a person given to meditation* (Oxford Languages, n.d.). This is the nature of these animals, encapsulated in the slow and forbearing ritual of heads turned downwards, browsing for bites of foliage; an undying attention to the ground.

Unlike other mammals, ruminants are able to digest fiber found in plant matter. Thus, ruminants are essentially in the business of converting sunlight into fuel that will nourish their forthcoming bloodline. Joann S. Grohman, experienced dairy cow owner and writer of *Keeping a Family Cow*, muses that "the only things that live lower on the food chain than cows and caterpillars are bacteria" 2013, p. *ix*), as they have the capacity to happily subsist on plant fiber only. Similarly, fungi are heterotrophic organisms, which rely on other organisms for food. Many fungi are considered decomposers, or saprotrophs, which release enzymes into their surrounding environments that decompose their food source in order to begin digestion. Others, like mycorrhizal fungi, form fruitful connections with a plant's root system, exchanging assistance with nutrient uptake for a plant's photosynthetic carbon.

Along with keeping themselves alive, these organisms perform extremely crucial functions in their ecosystems just by eating. Fungi make nutrients readily available by decomposing the world's endless supply of detritus, fueling the food web that all organisms are entangled in. They also support their ecosystems by increasing a plant's ability to withstand otherwise stressful conditions and promoting growth via mycorrhizal relationships (Bonfante & Genre, 2010). Cows translate the rough fibers of grass and hay into a rich, nourishing liquid composed of calcium, protein, and a plethora of fatty acids, like conjugated linoleic acid (CLA) and omega-3s (Grohman, 2013).

Cattle fertilize the ground they graze on by excreting their urine and nutrient-dense manure, stomping it into the earth while also mowing down less desirable weeds, thus encouraging native grasses to take over (Fountain, 2021). Their stomping motion ensures that seeds once unable to reach germination can flourish by mashing up the caked dirt that stifled them (Schwartz, 2013). This process has impactful results: the water retention rate of the land increases, especially important in drought-prone areas, while land erosion slows, causing decreased soil and nutrient depletion (Fountain, 2021). When cattle are managed effectively using rotating fencing systems, called intensive rotational grazing, the plant growth spurred by manure pounded into the ground is an effective means to sequester carbon via plant tissues able to store CO_2 (Fountain, 2021). All of this restorative action is a result of the cattle's wondrous rumen.

3.2 The Ruminant Digestive System

The ability of a cow to digest fibrous plant matter is made possible by optimized microbes housed in the rumen, one of the multiple chambers in a ruminant animal's digestive

system. This feat accomplished by these microbes is a delicate balancing act of chemical inputs and outputs. Two principal groups of bacteria are responsible for the digestion of the differing foodstuffs a cow may ingest. Those that aid in the digestion of fibers, like pasture grass, are most active when the pH in the rumen is around 6 to 6.8, nearly neutral (Grohman, 2013, p. 138). The saliva that a cow produces en masse during cudding, a process that involves repeated mastication, swallowing, and regurgitation in order to break down fiber, is alkaline and thus increases the pH of the rumen to aid in fiber fermentation (Grohman, 2013, p. 138). The breakdown of fiber by these bacteria contributes acetic acid to the cow, known as the source from which a cow gains her energy to produce milk. Bacteria that are adept at digesting plant proteins and starch, of which grain is the most typical, are most prolific and efficient when the rumen pH is more acidic, at approximately 4.5 pH (Grohman, 2013, p. 138). These bacteria aid with the synthesis of short-chain fatty acids, chiefly propionic and some butyric acid, which are transformed into the glucose that powers the cow's metabolism. Thus, acetic acid derived from fibrous plant matter contributes directly to milk production, while propionic acid synthesized from grain indirectly supports milk production by fueling basic metabolic ability (Grohman, 2013, p.139).

3.3 Ruminant Nutrition

Dairy cows are accustomed to a diet composed of some combination of the following: corn, grass, or rye silage, pasture grass, alfalfa, or grass hay, as well as some grain that acts as a metabolic supplement to a lactating cow. In theory, cows are able to subsist entirely on grazed or hay-derived fiber, but a cow producing milk, especially during the earlier stages of lactation, often needs a lot more caloric energy than can be physically provided by means of hay feeding

(Grohman, 2013, p. 137). Grohman (2013) notes that even if the cow had enough time to consume sufficient hay to energize her for the extremely intensive act of lactating, “without the extra energy in grain she may lose too much body weight” (p. 137). Grain is a supplemental aspect of the dairy cow’s diet; in fact, foregoing good hay for added grain in a cow’s diet, or feeding too much grain in general will only serve to fatten up the cow and in turn suppress milk production by creating the preferred acidic habitat for the starch-digesting bacteria in the rumen (Grohman, 2013, p. 139).

The dietary needs of a cow (a female bovine that has borne a calf or calves) and that of a steer (an infertile male bovine typically utilized for meat production) are varying. In addition to their requirement for plant fiber to give milk, dairy cows generally have a comparably discerning palate and sensitive diets due to the fact that what the cow ingests is directly related to the flavor profile of her milk. Especially if milk is unhomogenized, flavors resulting from the diet of a certain cow may be especially apparent to the milk consumer. Homogenization is a process that agitates and emulsifies milk, usually from many cows, in order to distribute fat globules and other particles, like dead bacteria, within the milk (Grohman, 2013, p. 9). When a cow comes down with an infection of the udder due to improper milking technique, called mastitis, her milk often emerges as stringy (Grohman, 2013, p. 24). In large dairies, homogenization helps incorporate this flawed milk into production. The dead leukocytes, or white blood cells, combating this infection that come out of the udder at milking time would otherwise create an undesirable texture and sediment to the milk if it was left unhomogenized (Grohman, 2013, p. 9). Thus, in smaller dairies especially, where homogenization can typically be excluded from the production process, it is vital to control for a cow's diet, for the sake of the milk as well as the health of the cow.

Crude protein (CP) is another vital aspect of cattle diet. Crude protein influences the growth rates of rumen bacteria that digest feed, so that inadequate levels of CP cause feed digestibility and intake to decline. Diets poor in CP translate to stunted muscle growth, reduced milk quantities in lactating cows, and improper reconditioning of the reproductive tract after calving (Parish & Rhinehart, 2008). Because CP is crucial to the health of cattle, protein supplementation is necessary when feeds have CP levels below 8%, as ideal levels of CP range from around 25% to 50% (Parish & Rhinehart, 2008). Supplementation is often achieved by incorporating protein blocks, liquid supplements, or high-quality forages, which are feedstuffs that have increased CP levels and low fiber content. Protein supplementation is expensive and often comprises most of the cost of supplemental feed expenses (Parish & Rhinehart, 2008).

3.3.1 The Role of Grain in Industrialized Beef Production

Generally, steers consume a lot more grain than dairy cows do, for the sole reason of increasing body weight for meat production. In larger beef farming operations, when calves reach about a year old, or about 900 lbs, these ‘yearlings’ are placed in feedlots. The diet of the cattle is progressively altered from grass and other forages to roughly 90% grain. This gradual dietary change is instituted so that the cattle can reach an appropriate commercial weight of approximately 1,300 lbs and their meat can take on a marbled, tender quality (Canadian Cattlemen’s Association, 2010). Grain not only increases the cattle’s ability to gain weight, but does so with unnatural speed: Weis (2013) reports that from the time they are born, feedlots cause calves to reach market weights appropriate for slaughter in as little as eighteen months (p.100).

Grain-finishing cattle, which refers to the practice of putting cattle in feedlots with grain-heavy diets to bring them up to commercial slaughter weight, replaced the archaic practice of solely grazing beef cattle (Fountain, 2020). This shift became normalized in the ‘60s, after the challenges of sufficiently fattening cattle in a timely manner due to limited winter feeding options and increased energy expenditure on open pasture became unprofitable to cattle farmers (Fountain, 2020). According to *The Ecological Hoofprint* by Tony Weis (2013), this change in livestock management can also be attributed to the mechanization of agriculture. Agricultural machines require uniform land use conditions to operate at financially viable scales, which is quite opposed to traditional mixed-use farms and grazing practices historically employed by farmers. Even with the advent of the McCormick reaper and the John Deere steel plow in the 19th century, farm animals were able to retain some of their role in farming. But as combustion engines became popularized, farm animals made way for machines. Once mechanized farming prevailed, along with innovations in fertilization, seed engineering, and pesticide use, concentrated feeding operations became a seemingly economical (and now, integral) means to utilize the grain and oilseed surpluses generated by these extremely high-yield and environmentally devastating subsidized farming methods (Weis, 2013). Weis (2013) coins the present relationship between concentrated feeding operations and the practices used to feed these animals as the "industrial grain-oilseed-livestock complex” (p.93).

The cycle that is perpetuated at the hands of this complex comes with a host of concerns. When it comes to feeding cattle with grain and oilseeds, one of these prevalent issues is land use. It is estimated that about half of the harvested acreage in the United States is dedicated to feeding livestock (Lappé, 2021, p.67). Grain feeding, especially at the scale that it is happening now, has proven to be massively environmentally detrimental. Soil erosion in the U.S. caused by major

animal feed crop farming practices is threatening the fecundity of farmland at an alarming rate, with fossil fuels being the main energy source for the production of these crops (Lappé, 2021, p. 10). Oilseed and grain crops are water-intensive, especially with drier and hotter temperatures. The irrigation of these crops often relies on underground aquifers, whose recharge rates are slowing with time (Lappé, 2021, p.78; Weis, 2013, p.108). Cattle, in particular, make 'poor' use of this ecologically intensive feed, as their feed conversion rates are the lowest compared to other livestock: about 16 lbs of grain feed are needed to supply a single pound of beef (Lappé, 2021, p.69).

Despite all of these negatives, proponents of industrial cattle farming often tout that the industry has decreased emissions relative to small-scale operations. One of their reasons is that diets high in grain, which are characteristic of factory farms (otherwise known as Concentrated Animal Feeding Operations [CAFOs]), reduce the level of methane emissions caused by enteric fermentation when compared to grass or hay-based diets (Beauchemin et al., 2008). The comparatively less-cellulosic nature of grain bypasses the fermentation process that occurs in the rumen to break down cellulose, which is the point at which methane is released as a byproduct when cattle consume grass or hay (Grohman, 2013, p.167). Industrial farming practices have also shortened the lifespan of modern-day cattle by speeding up the time that it takes for the animal to reach appropriate slaughter weight (Weis, 2013, p.100). This decrease in lifespan correlates to a decrease in methane emission, simply because the animal is alive for less time and will therefore emit less methane as it digests feed. These 'benefits', however, are far outweighed given the sheer amount of cattle being cycled through CAFOS. The claim of reduced methane emissions and increased feed conversion efficiency per animal becomes paradoxical given the resource

intensity needed to perpetuate industrial systems and the fact that there are simply more animals that are being cycled through them (Weis, 2013, p.115).

These tensions are only further exacerbated by rising meat consumption rates across the globe. The human population is expected to reach 9.8 billion by 2050 (United Nations, 2017). This is about a 3 billion increase from the world population in 2008. With this rise in population, meat consumption is predicted to disproportionately double from 2008 to 2050 (The World Counts, 2018), even though the population will not. This jump in consumption isn't only a symptom of growing populations– it can also be attributed to changing socioeconomic and cultural factors. Milford et al. (2019) report that there have been overall increases in income per capita in a majority of countries around the world, which is linked to the ability to purchase meat as it is generally more costly than other foodstuffs. As national income increases, as well as urbanization rates, so does the demand and consumption of meat (Milford et al., 2019). Thus, the rise in meat consumption is only partially correlated with population increase, as it occurs at a much faster rate than the human population will grow. It is important to note that despite increasing accessibility to meat products across the globe, wealthier countries like the United States will remain leading meat consumers in the coming years (OECD/FAO, 2021) and thus will be responsible for the emissions associated with it.

Utilizing a by-product like spent grains, especially if they can be enriched and tailored to cattle nutrition, has the potential to mitigate these dramatic environmental strains linked to cattle and meat consumption, at least to some extent. American livestock consume around 200 million tons of feed annually, most of which is grain and soybeans (Lappé, 2021, p.67). As mentioned earlier, spent grain is generated at an annual rate of 39 million tons globally (Lynch et al., 2016), with 10 million of those tons hailing from the U.S. (ReGrained, 2020). It is true that the spatial

dispersion between breweries and cattle farms will lessen the viability of transporting these spent grains directly to cattle, but it is clear that the amount of spent grain available is enough to enact even a slight structural transfiguration of the current "industrial grain-oilseed-livestock complex". This is especially true if the BSG can become tailored more closely to a cattle's rumen, even if the result may only be viable as a supplement in the cattle's diet.

As stated earlier, cattle are often cited as having the least efficient feed conversion rates when compared to other livestock. What might happen to the way we understand this 'poor' conversion rate if it was informed by feed composed of what otherwise might be considered a waste product? What if this waste product has the possibility to create more income and more calories via fungal growth alongside the nourishment of cattle? Frances Moore Lappé is famous for her seminal book *Diet for a Small Planet*, which discusses the moral implication of cycling grain into cattle that inefficiently convert it to meat in a world where many people are improperly nourished. Might Lappé still consider beef husbandry to be so wasteful if the manners in which cattle were fed could be more ecologically sound?

3.4 State of the Dairy Industry

3.4.1 Dairy Industry Consolidation

The tally of small and family-scale dairies in the U.S. has been consistently shrinking for the past 30 or so years. From 1997 to 2017, the U.S. experienced a 64% decrease in family-scale dairy enterprises and a corresponding 38% increase in milk production as factory dairy farming became enshrined in the playbook of American agricultural practices (FWW, 2023). As the factory farm's pervasiveness grew, so did the methane emissions associated with large-scale manure management. From the 1990s to 2020, methane emissions solely from dairy manure

management increased by two-fold, despite a mere 4.4 % increase in the actual amount of dairy cows producing milk (FWW, 2023). Dairy-producing factory farms often utilize waste lagoons. These lagoons accumulate manure and urine, creating a wet 'slurry' that is the ideal oxygen-starved environment for microbes to anaerobically break down the waste and release methane in the process (Hribar & Shultz, 2010).

The mismatch between the dwindling demand and ballooning supply of dairy products has introduced numerous governmental intervention programs over the last century. Famously, government buyouts of milk in the '80s and '90s due to overproduction forced the government to get creative with ways to keep milk from spoiling, leading to the creation of millions of pounds of 'government cheese' that was eventually distributed to low-income families or food banks through the Temporary Emergency Food Assistance Program (DiModica, 2021). The cheese was known for often being moldy. Other efforts to prevent overwhelming milk surpluses were the ubiquitous 'got milk' adverts of the early 2000s, as well as a government-funded bailout of Domino's Pizza in 2010[1] that was intended to keep the cheese-vending pizza chain in operation (DiModica, 2021). These government initiatives devised to keep the dairy industry afloat prioritize large agribusinesses over small farms. The dairy lobby that drives these economic contributions to the industry is extremely powerful, securing 43 billion dollars in milk buyouts from the federal government in 2017. Nearly half of the profit generated by dairy producers in the States in 2018 was comprised of government dollars, although the allocation of this revenue is chiefly oriented toward the large companies that power the dairy lobbies (DiModica, 2021).

[1] Domino's Pizza received 12 million dollars in funding from Dairy Management Inc. in 2010 to launch a marketing campaign, saving the company from declining sales that threatened the business. Following the partnership, Domino's pizza increased the amount of cheese on their pizzas by a whopping 40% in order to stimulate the dairy market by further promoting cheese as an integral part of the American diet. Even though Dairy Management Inc is not a direct branch of the USDA, the USDA both funds the association and elects some of its board members. Dairy Management Inc is a trade association that receives most of its funding from government fees on U.S. dairy products and federal tax dollars. Dairy Management Inc is also behind the famous 'got milk' advertisements (Moss, 2010).

The time before the early 2000s was an age when dairy policy in the States accounted for, albeit questionably, rife milk oversupply in order to reduce price fluctuation. Since then, policy protecting farmers from volatile milk pricing has been weakened, and much of the surplus milk generated by the industry is shuffled to export markets, leaving farmers vulnerable to the instability of unpredictable international markets (FWW, 2023). Additionally, there is a lower average cost associated with large dairy farms, and thus higher economic returns from milk production at this larger scale. This higher profit margin can be partially attributed to fundamental differences in milking practices (MacDonald et al., 2020). Small dairies often rely on family labor or a limited hired crew who milk twice a day. In contrast, larger dairies have an increased ability to hire more employees and are thus able to perform milking three times a day. In addition, technological advancements that are more accessible to large dairies have computerized the life of a dairy cow, streamlining her milk production as much as possible to achieve higher milk yields. These technologies include feed delivery systems that offer bespoke rations precisely adhered to a cow's lactation cycle and age, and automated milking systems that report data for each cow milking (MacDonald et al., 2020).

It was reported by Food and Water Watch (2023) that "the average U.S. dairy managed to turn a profit just twice between 2000 and 2021". This figure is deceiving, however, as the distribution of profit amongst the dairy industry is disproportionately skewed away from small-scale dairies, partially as a result of the manifold expenses associated with small dairying. Farms with small herd sizes saw negative net returns every single year from 2005-2018, while farms in the largest herd size range benefitted from positive net returns for all but four years in the same time period (MacDonald et al., 2020).

When small-scale dairies are unable to generate sufficient revenue from dairy alone, they are forced to either “get big, or get out”; meaning that they must choose between abandoning their livelihood or submitting to large-scale dairy practices by selling out or increasing their herd and operation size drastically (FWW, 2023). To put this consolidation into perspective, dairy farms that were considered large in the ’90s ranged from 100-199 head of cattle, and farms ranging from 10-199 head accounted for about 70% of milk cows in the United States. In 2017, the range of commercial farms with 10-199 cattle accounted for only 22% of milk cows. Now, the most common dairy farm size operates with 2,000 plus head, and this farm size is only becoming increasingly standard in the industry. These mega-farms don’t typically graze their cattle, nor are they often co-located with their own feed crops (MacDonald et al., 2020). Thus, they rely heavily on feed that is transported to them, further magnifying the worrisome environmental impact of the industry.

3.4.2 Income Diversification for Small Farms

Waiting for sufficient legislation to pass that may alleviate the financial pressures of small-scale dairying has not proved to be a successful enough tactic, as demonstrated by the dwindling number of small dairy operators across the country. Although it shouldn’t be the case, much of present action to better the condition of small dairy farmers has stemmed from small farmers themselves. One of these tactics is the incorporation of alternate income streams that are compatible with dairy farming but rely less on the volatility that informs profit accretion from selling plain milk. Income diversification for small dairy farmers may be instrumental to their survival in the face of ever-increasing production costs and industry consolidation.

One of the most common ways that a smaller dairy farm is able to generate non-dairy-driven income is through crop cultivation, typically grain. The ways that farmers utilize this crop vary; some farmers make the choice to sell the grain and others opt to use it to feed their cattle. This decision is fraught with unpredictability, however, as relying on grain may leave farmers vulnerable to incurring higher costs if their crops are less successful than expected due to weather variability, forcing them to purchase additional feed at higher prices from another party (Mahnken & Hadrich, 2018).

The Cornell Small Farms program published a collection of interviews in 2010 with New York State dairy farmers. Their aim was to disseminate successful strategies to remain profitable as small dairy farmers. Some farmers reported successes when they opened their businesses up to the public for tours and interactive events like hayrides, drawing in more customers and generating interest in their operations through what is termed ‘agritourism’ (Cornell Small Farms Program, 2010). Even if these tours are free, consumers will form a bond with the brand that the farm produces for. This brand loyalty translates to a higher likelihood that customers will purchase goods from the farm in the future, and recommend members of their social circles to do the same. Another successful tactic that may be merged with the agritourism model is direct marketing, in which a farm obtains a permit to sell its own goods on the farm. Cutting out the middleman, like a distributor, means reducing costs that a farm may incur to sell their goods elsewhere, like transportation costs or farmers' market participation fees. Direct marketing eliminates the need to sell farm goods at wholesale prices, so farmers can reap the higher returns of selling their products at retail prices. Additionally, it is profitable for dairy farmers to produce added-value products using their milk, like yogurt or cheese. Yogurt is an especially feasible

starting point, as there are relatively few barriers to entry and it is easier to produce than cheese (Cornell Small Farms Program, 2010).

3.4.3 Income Diversification and This Project

This project is a means to explore a novel avenue of income diversification for beef or dairy farmers. Taking inspiration from an interview from the Cornell Small Farms Program, income diversification can be most beneficial if the alternate enterprise works well in tandem with dairying or cattle husbandry, and not as a totally divorced operation. The introduction of fungi to a small-farm system may stimulate an advantageous partnership that can easily persist amongst preexisting farm infrastructure, favorable to both the farmer's wallet and the cattle themselves. If small farms are able to diversify their income and thus increase their ability to remain in the industry, disobeying the command to either "get big, or get out", the pace at which consolidation towards factory farming occurs might theoretically slacken. If income diversification became widespread and easily accessible, the myriad offenses of the factory farm may be repeated less often in our food system. The methods by which this project seeks to encourage income diversification– that is, by cultivating fungus to both supplement the cattle's diet and create edible, marketable fruiting bodies– may not be the perfect avenue to achieve a viable secondary income stream. However, it is important to continue the search for novel and appropriate solutions for the problems that our food systems, and the people that comprise them, face today.

3.5 Will Cattle Eat Brewer's Spent Grain Enriched with Mushrooms?

There have been plenty of studies pointing to the success of animal consumption of lignocellulosic and cellulosic materials enriched with fungal mycelium. One of these studies, conducted by Chuang et. al (2020b) purported a great benefit to broilers (chickens raised primarily to be slaughtered for meat consumption) when their diets were supplemented with spent mushroom substrate at a rate of 0.5%. Chuang and their colleagues used the dried waste product generated via the cultivation of *Pleurotus eryngii* (King Trumpet) on a substrate composed of *Pennisetum purpureum Schum* (Elephant Grass) as a feed additive. The substrate was collected for use in this experiment after the fungi had previously fruited and the fruiting bodies were harvested. The broilers exhibited improved fat metabolism, enhanced feed conversion rates, and increased antioxidant capacities (Chuang et. al 2020b). Wang et.al (2017) report similar results, where the substrates left over from mushroom cultivation were able to enhance antioxidant capacity in chickens. Antioxidant systems in organisms work as a defense mechanism from reactive oxygen metabolites, or free radicals, in order to decrease the body's susceptibility to diseases (Mandebvu et al., 2003).

Another study conducted by Bonanno and colleagues (2018) tested the effects of feeding sorghum grains myceliated with medicinal fungi to ewes for a period of ten weeks. The fungi made use of in this experiment included *L. edodes, Cordyceps spp., Ganoderma lucidum,* and *Pleurotus ostreatus*. The authors found that feed supplemented with 20% myceliated grain, their highest supplementation treatment, had numerous beneficial effects on the ewes compared to their counterparts receiving half that, or zero, myceliated grain. Some of these astonishing results include increased milk yields and milk casein content, dry matter and nutrient intake, and lesser incidence of intestinal parasitic infection. The feed was not only beneficial to the overall health

of the ewes but was also found to enhance the quality of the dairy products produced by elevating the antioxidant compounds present, thus heightening the oxidative stability of the cheese fat (Bonanno et al., 2018).

Results that pertain directly to cattle are slim and vary in success. In fact, there is only one available scientific paper that reviews the effects of fungal-treated biomass as cattle feed using an in-vivo treatment method. This lack of prior research that documents feeding trials done specifically with cattle persists despite ample literature on the subject of successful in vitro fungal lignin degradation of possible ruminant feed. The only readily available feeding trial experiment, conducted by Adamović and their colleagues (1998), demonstrated that wheat straw myceliated with *Pleurotus Ostreatus* became enhanced nutritionally with the addition of the fungus. Lignin content was shown to decrease, while crude protein content and free sugar contents were increased. These results are promising and indicate increased digestibility, however, the incorporation of this enriched feedstuff into cattle diet proved slightly challenging. In their 2-month feeding trial using this fungally enriched material, cattle rejected concentrations of the mushroom substrate in their feed exceeding 17%, and would only consume this much if incorporated with other more familiar feedstuff. The cattle fed myceliated straw compared to the control group saw slowed growth rates, unable to put on as much weight due to a reduction in voluntary feed intake (Adamović et al.,1998).

In this project, attempting to correlate markers of digestibility in cattle that are elucidated by a wet chemistry analysis with palatability and feed intake will serve to predict, not guarantee, the intake of the myceliated spent grain by cattle. It is most likely that if successful, the introduction of this enriched food product will be limited to supplementation, following the practices found to be viable by Adamović et al. (1998), especially if the cattle are already

accustomed to a specific diet. Grohman (2013) notes that cows are selective, sensitive creatures, and that any change in their diet is to be done gradually and with careful observation (p. 140). Grohman (2013) also reports that to stimulate the interest of cattle in a particular feedstuff, most probable on smaller scales, the incorporation of molasses or other suitable and tasty substances is recommended (p. 147).

4. Materials and Methods

4.1 Homebrewing

In the interest of having multiple spent grain samples to experiment with, a Hefenwezien beer homebrew kit was obtained from Northern Brewers. This kit came complete with grain, which will eventually become the spent grain used for this project. About 9 ounces of milled Briess Carapils grain comes with the kit, which is enough to send to the laboratory for forage analysis testing. Because the spent grain is obtained early and easily in the homebrewing process, additional milled Briess Carapils grain was acquired in order to generate sufficient quantities to innoculate with fungi.

The homebrewing process calls for 2.5 gallons of water to be heated to 76℃, at which point the grains can be added for steeping. The grains are put in a tied muslin bag, which is steeped in the water as it heats for approximately 20 minutes. Once 20 minutes have passed, the grains are removed from the liquid and are now considered spent. This process was repeated three times in order to generate enough spent grains for shipment to the forage analysis laboratory, to inoculate grain without sterilization (treatment), and to innoculate with the use of sterilization (control).

4.2 Inoculation of Spent Grains with *Pleurotus eryngii*

Brewer's spent grain obtained on March 2nd from Lasting Joy Brewery in Tivoli, NY, as well as the spent grain from homebrewing on March 13th were used in this study. Inoculation with *Pleurotus eryngii* liquid culture from North Spore occurred on the same date of collection for each spent grain sample. Quart-sized jars ball jars fitted with a DIY self-healing injection port as well as a hole covered with micropore tape to ensure fresh air exchange was used to collect the grains. Construction of these jars follows instructions from OneEarth Mushrooms (2021a). One of these jars was inoculated in a DIY still air box immediately after the grains were collected. The still air box is designed to mimic a laminar flow hood on a budget in order to prevent contamination during inoculation. It is built according to instructions from OneEarth Mushrooms (2021b), with some minor modifications. The transparent box is retrofitted with holes so that the user can insert their gloved hands into the plastic container. A loose seal is created by covering the holes with duct tape, and then cutting x's into them so that hands can fit through them. Isopropyl alcohol is sprayed in and around the still air box, as well as onto the empty jar. The freshly spent grains were immediately put into the box from the moment the beer brewing was finished and allowed to cool for a few minutes. The jar was filled with the grains, then inoculated with a *P.eryngii* liquid culture syringe through the self-healing injection port with 2 cc's of liquid inoculant, as is typical when using liquid inoculant for fungal cultivation (Shields, 2017c). The other jar was filled with spent grain outside of the still air box, strained of its excess liquid, and then sterilized in a pressure cooker at 15 psi for 90 minutes, as directed by Shields (2018). Once this sterilization is complete and the jar is allowed to cool, the jar is inoculated with liquid culture. The purpose of this second jar, the control, is to compare its mycelial growth with the jar full of unsterilized spent grains. This will determine whether the

pasteurization in the brewing process is functional within mushroom cultivation, or if it lends itself to contamination too readily without further sterilization. All of the inoculated jars are left to develop mycelia for a few weeks in a dark, warm, and slightly humid environment (around 18℃ and between 59- 70 % relative humidity). These inoculation methods were repeated with the spent grain obtained during the homebrewing process.

The grains obtained from Lasting Joy Brewery that were unsterilized and inoculated on-site were not strained, despite being significantly moist. After observing the delayed growth of the mycelium in this sample, which might have been due to the excessively high moisture content, an amendment to the methods of this project occurred. The spent grains obtained from the homebrewing process were strained by hand in the muslin cloth used to steep them while brewing, so that no water was left dripping from the grain. This was done using rubber gloves sterilized with isopropyl alcohol, immediately following their removal from the hot water after brewing in order to reduce the chances of contaminating the grain.

Following this point in the methods for this experiment, the writing regarding fungal cultivation outlines steps that were planned to be executed but were not completed in the duration of this project. The inability to move forward with substrate preparation and fungal cultivation was due to an insufficient amount of spent grain samples exhibiting mycelial growth that could be used for both fungal cultivation and nutritional analysis. Ultimately, the choice was made to use the viable sample for nutritional analysis rather than for cultivation purposes. Once the mycelium colonizes the grain sample, further cultivating the fungi follows well-established methods that produce relatively predictable results. This will be further discussed in the results and discussion section of the project. Nonetheless, the following may serve as recommended methods for fungal fruiting.

4.3 Pasteurization of Straw Substrate

Once the spent grains have become colonized with mycelium, the resulting material becomes known as grain spawn. Grain provides the mycelium with a nutrient-dense initial food supply, and once the mycelia is well-established, it can be incorporated with additional material, to 'spawn' more mycelia and create colonization of a larger area. The material that the grain spawn is integrated with is called bulk substrate. Because the mycelia at this point in its growth tends to be firmly established, bulk substrates are typically less nutrient dense and cheaper, oftentimes being composed of waste products like sawdust or cardboard. Bulk substrate can be pasteurized instead of sterilized, eliminating the energy and cost intensity of sterilization practices. This is on account of the vigorous nature of the grain spawn, which is now less susceptible to contamination by other organisms, and will quite swiftly overtake the novel substrate once the two are incorporated.

Pasteurization methods for bulk substrates commonly prescribe heating submerged material in hot water. On a small scale, this process might not seem terribly impractical. However, the same cannot be said for larger operations, or production systems which require low-energy and low-cost inputs. Cold water lime pasteurization can be used to evade these costly inputs, although it is only effective for a select few substrates, chiefly straw. It is a process by which hydrated lime, or calcium hydroxide $Ca(OH)_2$, is added to water in order to increase the pH level. The shift towards alkalinity occurs swiftly and is quite pronounced, which makes conditions unsuitable for most bacterial or fungal life that might compete with the desired mycelia (Shields, 2018).

Cold water lime pasteurization in this project was intended to be completed following guidelines from FreshCap.com, a mushroom cultivation company and blog by Tony Shields.

Materials to be utilized included a 5-gallon bucket and lid, a pillowcase, hydrated lime, a kitchen scale, rubber gloves, and a face mask. Once geared up with personal protective equipment, as lime can be hazardous to breathe in, hydrated lime should be added to a bucket filled with cold water. Six grams of lime need to be added for every gallon of water utilized, although it is stressed by Shields that these measurements do not have to be exact. In order to ensure that there was sufficient room for the straw, the 5-gallon bucket should be filled with 4 gallons of water and 24g of hydrated lime. Once the alkaline solution is prepared, 10 quarts of straw should be added to a pillowcase, then placed in the bucket. A brick over a small cooking pot top can be used to weigh the straw down in order to ensure total immersion.

The pH level the straw bath is expected to reach in order to be effective enough to pasteurize the straw is anywhere between 11-14 pH (Sayner, 2022b). Using an inexpensive pH meter can be an effective measure to ensure that the lime is successful in creating alkaline conditions.

The straw can be soaked in the lime solution for 12-24 hours according to Shields (2018). When the straw is ready for removal, it is placed on a clean mesh screen to dry for 20 minutes. The straw is too wet to be used immediately after this process, so drying is necessary. However, exposing the straw to open air for more than 20 minutes will give airborne organisms another chance to potentially contaminate the straw.

As mentioned above, mushrooms are able to subsist on some pretty unique substrates. Initially, the intent of this project was to solely utilize waste products, so the chosen bulk substrate to accomplish this was originally sawdust from the student woodshop at Bard. Once the quality was assessed of this by-product, however, it was determined that the sawdust was too fine and variable in composition. Sawdust, especially sawdust of an extremely fine nature, is difficult

to dry enough to optimal moisture levels as it retains much of the water used in cold water lime pasteurization methods. Because the spent grains used to create the grain spawn are also quite high in moisture, it is best to utilize a substrate that won't be oversaturated with water.

Ultimately, chopped straw was chosen to be the bulk substrate intended for use in this project for a variety of reasons. In addition to being inexpensive, straw responds quite positively to cold water lime pasteurization methods. It is also an extremely successful and commonly utilized substrate for the growth of Pleurotaceae family fungi. Because utilizing brewer's spent grains to foster the growth of mycelia is already a potentially finicky process (relative to the expected growth under optimal conditions), I opted to stick to straw for its reliability.

4.4 Substrate and Spawn Bulking

Once the straw has been pasteurized, it is ready to be incorporated with the grain spawn. Similar to inoculation of the grain jars, this process also requires conditions that are as clean as possible. It is less important to be completely sterile here because the mycelia will already be well-established and vigorous, though it doesn't hurt. The DIY still air box can be utilized once again for this procedure. Other materials include gloves, isopropyl alcohol sanitizer spray, mushroom grow bags, and zip ties. The grow bags are clear, 3.0 mil thick, polypropylene plastic bags that can hold about 12 quarts of total material comfortably.

Working in layers, straw and grain spawn should be pushed down into the plastic grow bag. Straw substrate is inoculated at a rate of 10-15% with the grain spawn, according to methods put forwards by Shields (2018). One quart of grain spawn can be utilized for just shy of 10 quarts of straw. Once the bags are filled and tied off with a zip tie, leaving some space between the top of the substrate and the seal, the bags should be cut with small vertical slits that are about 10-15cm apart. These slits should be evenly spaced, and cutting the top or bottom of

the bag should be avoided (MycoLogic, n.d.). This is so that proper fresh air exchange can be achieved during the growing process and excess levels of carbon dioxide will not be trapped inside of the bags. Mycelium typically grows beneath the first layer of soil, underground, so it can withstand some level of carbon dioxide concentration. However, fresh air exchange is crucial to fungal cultivation, as fungi require oxygen intake and CO_2 release during their development (Sayner, 2022a). Creation of these slits is also to allow space for the fruiting bodies to develop later on.

4.5 Pinning and Fruiting

After the straw has been inoculated with the grain spawn, the mycelium needs to further overtake the substrate in preparation for fruiting. Optimal conditions for this are a warm environment, anywhere between 16-28℃ (MycoLogic, n.d.). It is important to maintain a moist environment, which can be done by surrounding the grow bag with a dark plastic trash bag and spraying water around the bag periodically. The relative humidity should be maintained around 95% (Shields, 2017a). Monitoring of the humidity can be achieved by the use of an inexpensive hygrometer. This process can take anywhere between 7 and 20 days, depending on how ideal the conditions are and how much grain spawn was used to innoculate the straw (MycoLogic, n.d.).

Once the straw is completely colonized by the mycelium, it is time to initiate pinning. Pinning refers to the small, bud-like formations that mycelia form as precursors to fruiting bodies. The grow bag should be placed in a location with lower temperatures than where it was colonizing, ideally around 15°C (Shields, 2017a). In this experiment, I was planning to place the grow bag in an outdoor shed to simulate these conditions. The area should be shaded and have some fresh air flow. Once ‘pins’ form, the humidity can be reduced to around 80%. This is

especially crucial when cultivating *P.eryngii* as it is a fungus susceptible to bacterial blotch that infects and darkens the fruiting bodies. This occurs when humidity levels are too extreme and water droplets are allowed to remain on the fruiting body for too long. Fruiting bodies should develop between 4-8 days after pinning is visible (Shields, 2017a). After the fruiting bodies create an elongated stem and flattened-out cap, the fungi are ready to be harvested. Harvesting time depends on the preference of the cultivator; harvesting earlier generally makes for more flavorful mushrooms but smaller yields, while waiting until the fungi fully develop ensures stems will be large and fleshy. Like with most other mushroom species, it is often possible to get a second 'flush', or harvest, of mushroom fruiting bodies when cultivating *P.eryngii. P. eryngii* fruiting bodies are also known to have longer shelf lives in the refrigerator when compared to other oyster mushrooms, lasting for about 2 weeks (Shields, 2017a).

4.6 Nutritional Analysis

On the date of initial grain collection, both from Lasting Joy Brewery and from homebrewing, a portion of the fresh BSGs were collected in a sample collection bag and sent to Dairyone Laboratory for nutrient analysis testing using wet chemistry methods. After the inoculated spent grains went through sufficient spawn running, or became colonized by mycelium, a sample collection bag of this material was also sent to Dairyone Laboratory for nutrient analysis. The sample is collected before the fruiting stage, as this is when the most lignin is broken down in relation to how much cellulose and hemicellulose remains intact (van Kuijk et al., 2015a). After fruiting, the fungi utilize some of these other fibers in their development, making the resulting feedstuff less nutritious for the ruminant.

The only treatment that generated sufficient mycelium to be sent to the lab for testing was the grain sample that was obtained from Lasting Joy Brewery and sterilized before fungal inoculation. In total, there were three samples sent to Dairyone Laboratories for forage analysis: two grain samples sent from each source, Lasting Joy Brewery, and the homebrew, and one sent after the grain from Lasting Joy Brewery successfully myceliated. This sample exhibited substantial mycelial growth, yet did not reach total mycelial colonization in the time allotted for this project. By the time it was appropriate to send the grain to be analyzed, the jar was about a quarter of the way populated by mycelium (Fig.1).

The nutritional qualities measured in this analysis include Moisture Content, Crude Protein (CP), and Neutral Detergent Fibers (NDF). In order to quantify how much lignin is broken down after *P. eryngii* is allowed to colonize the spent grains with mycelium, Neutral Detergent Fibers (NDF) will be measured both before and after inoculation. NDF is a measure of fiber in the cell wall of the feedstuff in question, representing the cell wall's composition of lignin, cellulose, and hemicellulose (Belyea et al.,1993). NDF is considered a marker for how rapidly ruminants will become full of the feedstuff in relation to how much of the actual feed is consumed. A feed that is high in NDF will quickly fill up a ruminant's stomach with a reduced amount of food, resulting in decreased milk production (Rosales et al., 2022). NDF is a good indicator of the level at which a ruminant will intake a certain feed (Belyea et al.,1993). Following a hypothesis proposed by van Wyk (2021) when determining the ability of fungi to degrade lignin, NDF contents in spent grain should exhibit a decrease as *P.eryngii* colonizes the BSGs.

5. Results and Discussion

5.1 Nutritional Value of Spent Grains Enriched with *P. eryngii*

Table 1. Results of BSGs analysis from Dairyone Laboratories given as Dry Matter (DM) intake. Myceliated Homebrew grain entry is listed as n/a because it was not sent to the lab for analysis, due to lack of mycelial growth on the sample. The last entry in the table denotes the expected quantities of each nutritional parameter for BSGs according to references.

Sample Origin	Sample Type	Moisture Content (%)	Crude Protein(%)	NDF (%)
Lasting Joy Brewery	Fresh BSG	76.9	17.3	29
Lasting Joy Brewery	Myceliated	76.5	17.1	35.1
Homebrew	Fresh BSG	65.6	11.5	11.5
Homebrew	Myceliated	n/a	n/a	n/a
Average Brewer's Spent Grain		70-81[a]	26[b]	54.7[c]

Note: Data is from Thomas & Rahman (2006); Mussatto et al. (2006); Lynch et al. (2016)[a] , Parish & Rhinehart (2008)[b], Mad Barn (2019)[c].

The moisture content of the spent grains obtained from Lasting Joy Brewery was slightly outside of the range that the Pleurotus mycelium are able to tolerate (Table 1), which is between 50 to 75% (Bellettini et al., 2019). The homebrew sample was well within that range of tolerable conditions, however, the gluey and dense texture proved difficult for the mycelium to navigate.

The Crude Protein (CP) levels exhibited by all of the grain samples, including the myceliated sample, were slightly lower than what is expected from spent grain (Table 1). This is likely due to the type of grains used in both of the brewing processes. As mentioned earlier, ideal CP levels for cattle nutrition lie within the range of 25% to 50% (Parish & Rhinehart, 2008). The slight decrease in crude protein content exhibited by the myceliated grain sample is minimal, and thus requires further replication to confirm whether or not this can be considered an anomaly.

The NDF contents of all of the grain samples were well below what is expected for BSGs (Table 1). Again, this may be attributed to the type of grain utilized by the brewery and the homebrewing process, as variation within the category of spent grain is common. One of the aims of this project was to harness the abilities of fungi to degrade lignin in BSGs in order to achieve optimal levels of NDF in cattle feed. It was expected that the spent grains analyzed in this project would have elevated levels of NDF, thus introducing a problem that fungi may be fit to ameliorate. Interestingly, the spent grains from Lasting Joy Brewery tested in this experiment had NDF levels close to acceptable ranges for cattle feed, which are between 28% and 34% (Willoughby, 2022).

The NDF of the spent grains exhibited an increase from 29% to 35.1% after colonization with mycelium (Table 1). This result contrasts with the literature surrounding general fungal degradation of lignin, as well as literature describing the specific ability of *P.eryngii* to degrade lignin. This demonstrates that more replications are necessary to gain a better understanding of the factors at play in this experiment. It is possible that the level of mycelial colonization achieved played a role in this result, as literature on the subject suggests that it is necessary to have a completely colonized sample in order to obtain relevant results (van Kuijk, et al., 2015b). Because of this, additional replications and lab analyses at multiple stages throughout mycelial development may serve to illuminate at what point the sample is most viable in relation to cattle nutrition. Despite being an unexpected result, the NDF content after partial mycelial maturation still remains nearly within the suitable measures of NDF for cattle feed.

5.2 Effectiveness of Spent Grain in Fungal Cultivation

5.2.1 Pasteurization, Moisture, and Growth Rate

The most effective means of cultivating fungi in this study was exhibited when the grains from Lasting Joy Brewery were sterilized and drained before inoculation (Fig.1). It seems likely that the establishment of mycelium, in this case, was due to the reduced moisture content of the sample via straining before inoculation or due to the sterilization the sample underwent. It is

Fig. 1. Mycelial growth on sterilized BSGs 50 days after inoculation with fungal liquid culture.

difficult to determine, however, whether sterile conditions or moisture content played a larger role in the successful establishment of the mycelia. Because the other sample obtained from Lasting Joy Brewery that was directly inoculated without sterilization or straining exhibited very minor mycelial growth (Fig 2.), it is possible that the factor limiting mycelial growth was more so the extremely wet nature of the unsterilized sample and not the fact that it was not sterilized.

Fig. 2. Minor mycelial growth on unsterilized and unstrained BSGs from Lasting Joy Brewery 50 days after inoculation with fungal liquid culture.

It is important to note that no contamination is visible on any of the four samples. This doesn't mean that other microorganisms are not present, although it does indicate that on some level, there is not severe contamination to the point of visible mold formations. This is true for both sterilized and unsterilized grain jars, indicating that it is possible that the brewing process is able to pasteurize the grains enough so that immediate collection is able to prevent contamination

from microorganisms. This might be another signal that the moisture content of the grains is the factor that limited mycelial growth the most, as opposed to sterilization.

Fig. 3. Spent grains from the homebrewing process 35 days after inoculation with fungal liquid culture. From left to right: sterilized homebrewing sample, unsterilized homebrewing sample.

The grain samples derived from homebrewing were both strained of excess liquid, and one sample underwent pressurized sterilization, while the other was directly inoculated. Neither of these samples exhibited any visible form of mycelial development (Fig. 3). This may be due to the undesirable texture of the grain. The grain was finely milled and extremely compacted and mushy, forming an almost oatmeal-like texture that might have made it difficult for the mycelium to traverse and spread throughout the material. The grain obtained from Lasting Joy Brewery had

more intact, whole grain kernels than the homebrew grains, probably due to the fact that the grain blend used to brew the beers was not the same.

The rate at which the *P.eryngii* mycelium colonized the grain was much slower than under normal circumstances, that is, utilizing fresh, sterilized grain. The expected time frame for mycelial colonization of grain depends on the species of mushroom and the growing conditions, though it typically ranges from 1-3 weeks (Shields, 2017c). The grain that was obtained from Lasting Joy Brewery was inoculated on March 2nd, and did not achieve total mycelial colonization by the time it was required to be sent to the laboratory for analysis for completion of this project, over 7 weeks later. Based on the growth rate of the mycelium, I would infer that the grain would have taken about 4 more weeks to achieve total colonization. Fig. 1 displays the level of mycelial growth 50 days into the experiment, which was the same day that the sample was sent to the laboratory for analysis. For reference, Fig. 4 shows the level of mycelial growth that a fully colonized grain jar is expected to reach before it is added to the bulk substrate for further colonization. The process of mycelial growth can be sped up by increasing the amount of inoculant used (van Kuijk et al., 2015a). I considered doing so in this project, however, I chose to follow the established practices for *P.eryngii* cultivation to determine how comparable the methods are to one another.

Fig. 4. Total mycelial colonization of a grain jar.
Source: Milliken Mushroom Supply, (2022)

5.3 Other Limits of this Study and Future Improvements

Ultimately, this study would have benefited greatly from an increased number of replications. More replications of each treatment would add statistical significance to the results, painting a clearer picture of the general viability of cultivating mushrooms on spent grain as well as the effect that the mycelia have on the nutritional value of the grain. With more data points, it would become easier to isolate which treatment types are more effective than others, and why that might be, thus leading to confident recommendations for real-life applications of a system like this.

Due to cost and time constraints, the creation of more replications was not possible in this study. It is specifically important to highlight the cost of liquid mycelium culture, which may be a barrier to entry for future permutations of this concept. However, most mushroom cultivation operations are able to create their own liquid culture or agar plates which reduces the cost of mushroom cultivation if it is to be done at larger scales. The growing popularity of mushroom cultivation, as well as the increasing recognition of the benefits that fungi can have on food systems, may eventually reduce the cost of entry into fungal cultivation.

A recommendation for further strengthening the statistical significance of the results of this project may also include not only more replications of each treatment, but also an increased number of lab analyses performed on the spent grain as the mycelium colonizes the grain over time. Data points indicating the nutritional qualities of the myceliated spent grain at multiple developmental stages would serve to elucidate the precise point in the growth process that mycelia make the spent grain most appropriate for cattle feed, in terms of both CP and NDF contents. If it is true that some types and preparations of brewer's spent grains will take longer than usual to colonize with mycelia, as observed in this study, it is then necessary to measure the

effect of time on the quality of nutrition of the spent grain. Understanding the temporal variation of the spent grain would likely be an important aspect for the farmer to consider when utilizing the feedstuff. This is a practice cattle farmers are accustomed to when selecting forage, as the nutritional qualities and cost of the forage are largely based on its maturity (Nelson & Moser, 1994).

The initial draw of this project arose from the possibility of tapping into a preexisting system that might be conducive to fungal growth. A key principle of this is the idea that the grain will have already gone through pasteurization, which takes some of the cost and energy constraints related to mushroom cultivation out of the equation. Because it is ambiguous whether or not the lack of proper sterilization was the limiting factor to mycelial growth, or the moisture level of the grain, it is hard to thus recommend beer brewing as the ultimate and ideal precursor to fungal cultivation. However, because it is possible that the overly moist nature of the grain was the proprietary issue, it may also be worthwhile to control for this aspect of the process going forwards.

There may be easily applicable solutions at the level of the brewery that will reduce the water content of the spent grain, like straining the wort out of the grain more thoroughly. In the homebrewing processes, after the grain is finished steeping, it is imperative to avoid squeezing the grain bag to strain it directly over the wort before removing it from the liquid. This is because the extra sedimentation that seeps through the muslin cloth upon straining will end up in the beer and cause cloudiness or debris to settle at the bottom of the beer later on. This might be the same for brewing in larger contexts. When straining the grain bags from excess moisture in the homebrew process, it was done over a separate container, so that the grain was less wet but the resulting liquid from straining debris didn’t end up in the beer. To scale this up, brewers might

strain their wort from the spent grain in an initial flush, following their normal methods. Next, they might strain the spent grains one final time in order to reduce the moisture content, and dispose of the extra, lightly sedimented liquid that emerges from that process. It is also possible that different brewers spent grains from differing sources may have different moisture contents, and finding solutions may be more tailored to specific operations rather than the general idea as a whole.

A facet of this field of research that contributes to uncertainty is the surprising scarcity of formalized feeding trials, in which cattle specifically are subjected to diets incorporating fungal substrates. The one study (Adamović et al., 1998) that attempts fungal-based cattle feeding was performed almost 25 years ago. In that time, there have been considerable changes to the cattle industry. Besides becoming a leading greenhouse gas emitter in the agricultural sector, the genetic makeup of cattle in the United States is perpetually being altered via genomic selection and breeding, most notably to increase production traits (Guinan et al., 2023). This presents an opportune niche in which projects like these may have a place in the ever-changing landscape of the cattle industry. It may well be possible that differing cattle breeds will accept fungal-based feeds more readily, which would be a welcome cost and energy-saving endeavor for an industry that is in need of just that.

6. Conclusion

Fungi embody and enact circularity. Harnessing their abilities towards the improvement of anthropogenic agricultural systems means grappling with the intricacies of fungal development and cultivation techniques that may actively evade these very notions of organization. We see that fungi are both compatible and integral to the complicated, polycultural

systems that make up this planet. What is yet to be determined is the feasibility of introducing fungi into human-made systems in a way that might honor their tendency for inter-organismal connection. This project has not served to highlight the overwhelming successes of *P.eryngii* as a component of either cattle diet or of farmer income diversification given the parameters of the experiment. It has, however, served as a demonstration of the potential of *P.eryngii*, in that there are clear indicators of how further advancements may take shape. The ability of *P.eryngii* to develop mycelia on Brewer's Spent Grain in non-laboratory conditions has been exhibited to an extent that holds promise for future endeavors. For example, it might be possible that utilizing spent grains solely for fungal cultivation may be a more attractive alternative than attempting to incorporate the resulting material into cattle diets. It may also be possible that with further experimentation, fungal cultivation on spent grains and subsequent usage of the material as feed will become easily attainable. The sheer diversity that comprises spent grains, as well as growing conditions that fungi may endure, make for many possibilities to replicate this study, or conduct similar experimentation, and come away with differing results. This diversity is echoed in the needs and functional capacities of breweries and farms alike, where each operation is different from the next and thus require bespoke solutions. Whatever the means may be, prioritizing re-use and resource sharing within food systems is a highly achievable and extremely necessary goal for the future of our food.

At its core, this project is a call to enrich food systems so that their circularity becomes a tool to resist industry consolidation and monoculture. If these tight-knit systems can be both ideologically and financially attractive to small producers, there will be less reliance on powerful industry players by both producers and consumers alike. However, disrupting prevailing patterns of waste, power consolidation, and even notions of which organisms we privilege in our food

systems will require consistent experimentation and a willingness to adapt to novelty, and at times, to uncertainty. The unexpected results of this project are a testament to the complexity that defines circular food systems. Generative partnerships between unlikely organisms will comprise these systems, forming tangled webs that prove difficult, but worthy, to unravel. These partnerships must be concealed beneath the fast and dirty mechanisms of modern agriculture in order for the industry to exist. But if we care to look, they persist right in front of us, flourishing on the barren desert floor.

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