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Use of faba bean (*Vicia faba* L.) hulls as substrate for *Pleurotus ostreatus* – Potential for combined mushroom and feed production

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ABSTRACT

A circular concept involving use of faba bean hulls for production of oyster mushrooms (*Pleurotus ostreatus* (Jacq.) P. Kumm.) and the post-harvest residues for feed purposes was evaluated. Faba bean hulls are a waste remaining after the beans are dehulled to decrease the content of anti-nutritional factors and increase the food value of the beans. Faba bean hulls proved very suitable as substrate for production of oyster mushrooms, with biological efficiency of 109 ± 28%. The fruiting bodies produced were comparable to commercially sold mushrooms in terms of morphology, protein content, protein quality, and chemical composition. After mushroom harvest, 48.4 ± 0.6% of initial dry weight (dwt) of the substrate remained and showed significant changes in proximate composition, with an increase in protein concentration from 207.9 ± 8.6 to 346.6 ± 16.5 g kg^{-1} dwt and a significant increase in 14 of 16 amino acids analyzed, including methionine. Concentrations of the anti-nutritional compounds vicine and convicine were below the detection limit after mushroom harvest, while their initial concentration was 5.7 ± 0.1 and 1.4 ± 0.04 g kg^{-1} dwt, respectively. Tannin concentration was reduced by approximately 50%, to a final level of 9.0 ± 1.6 g kg^{-1} dwt. Comparison of the spent mushroom substrate with a commonly used feedstuff for pigs indicated potential for the spent substrate to be a valuable protein source in pig diets. This study demonstrates the potential for achieving complete circular use of agro waste and has implications for development of production systems well suited in the biobased society.

1. Introduction

A change in diet is proposed to help resolve major societal challenges, with increasing consumption of legumes identified as a strategy for sustainability in the food system (Röös et al., 2018). Despite this, production of legumes in Europe is low. In 2016, the area of arable land dedicated to grain legumes in Europe was only 1.5%, compared with 14.5% globally (Watson et al., 2017). Improving the market value of legumes, e.g., by using different types of processing techniques, is a recognized strategy that could promote production (Preissel et al., 2015).

Faba bean (*Vicia faba* L.) is a versatile legume crop that can be produced in different climate zones (Jensen et al., 2010). In addition to high nutritional quality, faba beans have high capability for nitrogen fixation, thereby reducing the need for nitrogen fertilizer (Multari et al., 2015). However, as with several other legume crops, there are anti-nutritional factors of concern that impair their nutritional quality. In faba beans, condensed tannins are a major anti-nutritional factor, with the highest concentrations of these substances found in the hull (Sinha and Kumar, 2018; Helsper et al., 1993). Thus, dehulling is an obvious strategy to increase the utility of the beans in different food products.

Dehulling results in hulls as a waste product and, considering the importance of circularity in food and feed production, there is a need for innovative ways to recycle this residue back into production. One possibility is to use the hulls as substrate for production of edible mushrooms. Commonly cultivated mushrooms can broadly be divided into two groups, compost mushrooms (secondary decomposers) and wood-degrading mushrooms (primary decomposers). Compost mushrooms, such as white button mushrooms, use partly degraded plant material for growth, while wood-degrading mushrooms, such as oyster mushrooms and shiitake mushrooms, can be grown directly on plant material rich in lignin and cellulose (Grimm and Wösten, 2018). Important characteristics of the substrate are a suitable carbon/nitrogen ratio and a physical structure that allows gas exchange during fungal growth (Stamets, 2000). Oyster mushrooms (*Pleurotus* spp.), are of high interest when

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Abbreviations

BE	Biological efficiency, meaning capability of a specific mushroom substrate for production of fruiting bodies of a specific fungal strain
cfu	colony forming units
dwt	dry weight
NDF	Neutral detergent fiber
SMS	Spent mushroom substrate
std	standard deviation
TN	Total nitrogen
WSC	Water-soluble carbohydrates

considering faba bean hulls as substrate for production of edible mushrooms, as they are primary decomposers well-known for their capability for fast growth and production of fruiting bodies on a wide array of different substrates (Fernandes et al., 2015). Both the legumes and the mushrooms in this system are important as a future protein source, with protein levels of 20–25% of dry weight (dwt) (Kalac, 2013).

Production of oyster mushrooms involves four different stages (Sánchez, 2010). First, the substrate is pretreated, often by pasteurization, to reduce the natural microflora, and inoculated with oyster mushroom spawn. The fungi then colonize the substrate through mycelial growth. The colonization results in degradation of the lignocellulosic content of the substrate due to fungal release of extracellular degrading enzymes (Eichlerova et al., 2000). When the substrate is fully colonized, initiation of fruiting body formation (fructification) is stimulated, often by exposing the substrate to an environment change such as a temperature decrease. Following successful fructification, the fruiting bodies are harvested in several flushes over time, leaving spent mushroom substrate (SMS).

The SMS remaining after mushroom production is composed of mycelium and partly degraded plant material, and may have several applications (Rinker, 2017). With a combination of faba bean hulls and oyster mushroom residues, considerable protein levels can be expected in the SMS. Therefore, its use as a feed ingredient is of high interest, and could contribute to decreased competition between human food and animal feed and lower dependence on imported protein feeds in the European Union.

The wide range of substrates that can be used by *Pleurotus* spp. and their fast growth rate is due to release of powerful degrading enzymes into the substrate (Eichlerova et al., 2000). These enzymes include different types of peroxidases, such as manganese peroxidase, and phenol oxidases, such as laccases. Formation of hydrogen peroxide and free radicals has been demonstrated to be highly important during the degradation process (Rhodes, 2014; Munk et al., 2017). The enzymes have low substrate specificity, which allows them to act on a wide range of complex molecules. For example, significant degradation of the anti-nutritional factor condensed tannins has been reported in plant material after growth of *Pleurotus* spp. (Espinosa-Páez et al., 2017).

The present study examined the potential for using faba bean hulls as substrate for production of oyster mushrooms. The quality of the mushrooms produced, including protein level, amino acid composition, and chemical composition, was determined. The SMS was analyzed with regard to its proximate and chemical composition and content of anti-nutritional factors (condensed tannins, vicine, convicine). Based on the values obtained, use of the SMS as a feed ingredient in pig diets was evaluated. As mushroom cultivation is an aerobic solid-state fermentation process performed at room temperature, the risk of establishment of food-borne pathogens in the SMS was also studied.

2. Material and methods

2.1. Fungal strain and mushroom substrate

Grain spawn of the oyster mushroom strain *Pleurotus ostreatus* M2191 was obtained from Mycelia BVBA, Belgium. Dried hulls of faba bean (*Vicia faba* L.) from the colored-flower cultivar 'Alexia' were obtained from a grower in the South of Sweden (Bottna, Bohuslän).

2.2. Mushroom production

The dried hulls were rewetted with distilled water to a moisture content of 70% and packed in boxes suitable for mushroom production (Sac O2, Nevele, Belgium). The pH of the substrate was 6.5. A total weight of 0.6 kg of substrate (wet weight) was packed in each box. The bags were pasteurized at 65 °C for 8 h and, when the substrate had cooled down, spawn of *P. ostreatus* was added in a concentration of 10% by dwt. Three boxes were used for each treatment and the whole experiment was repeated. The boxes were incubated at 22 °C with the lids closed for 19 days, by which time the substrate was densely colonized with mycelium. The closed boxes were then incubated at 10 °C in a refrigerator for three days to induce fructification, followed by removal of the lids and incubation in a climate chamber at 22–24 °C and humidity 85% until harvest of the first flush of fruiting bodies.

2.3. Analysis

2.3.1. Mushroom production and quality of the fruiting bodies

The amount of mushrooms (fresh weight and dwt) produced in the first flush was determined. The dry weight was recorded after lyophilization. Mushroom production (fresh weight) was related to the amount of substrate (dwt), in order to determine the biological efficiency (BE) of the substrate according to Equation (1).

$$BE = (\text{mushroom (fresh weight)} / \text{substrate (dwt)}) \times 100 \quad (1)$$

Total protein content in the fruiting bodies was analyzed by the Dumas method (Bellomonte et al., 1987), using a Vario Max CN and a conversion factor of 4.38 for total nitrogen (TN) (Barros et al., 2008). Amino acid composition, including alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, and valine, was determined at a certified laboratory (Eurofins Food & Agro Testing Sweden AB, Linköping, Sweden) by ion exchange chromatography (IEC) according to an existing method (Llames and Fontaine, 1994). To determine the chemical composition of the harvested mushrooms, the dried samples were wet-combusted in HNO_3 (65%) using a microwave technique (CEN Mars 5) and analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES).

2.3.2. Proximate analysis, amino acid composition, and chemical composition of the substrate

Proximate analysis of ash, total protein, neutral detergent fiber (NDF), water-soluble carbohydrates (WSC), and neutral lipids was performed on faba bean hulls before and after mushroom production. Before analysis, the dried biomass was milled (KG40; DeLonghi Appliances, Casula, NSW, Australia). The ash content was determined by incineration at 550 °C for 3 h, and cooled in a desiccator before weighing. A factor of TN x 6.25 was applied to determine total protein content. NDF was analyzed according to Mertens (2002), using an amylase neutral detergent method. WSC was analyzed with an enzymatic method described by Larsson and Bengtsson (1983). Lipid analysis was performed using an extraction system (Soxtec System HT 1043 Extraction Unit, FOSS Analytical A/S, Hilleröd, Denmark), without acid hydrolysis according to the manufacturer's recommendations (ANKOM Technology, Macedon, NY, USA), with modifications by Hooft et al. (2011). Amino acid composition and

chemical composition were determined as described above. Net energy values for SMS were calculated based on the results of proximate analysis, using EvaPig® (version 1.4.0.1).

2.3.3. Analysis of vicine, convicine, and condensed tannins in spent substrate

Condensed tannin content was analyzed by thioacidolysis as described elsewhere (Mattila et al., 2018). In brief, condensed tannins were depolymerized by methanolic HCl in the presence of cysteamine, and the reaction products were determined by high performance liquid chromatography with diode array detector and fluorescence detector (HPLC-DAD/FLD; Agilent 1100 series). For analysis of vicine and convicine, the samples were extracted with water in a hot water bath for 3.5 h. Concentrated HCl was then added to the sample extracts, which were analyzed by HPLC-DAD. The breakdown product divicine (and/or isouramil) was tentatively identified by its UV spectrum, and quantified using the calibration curve for vicine and correcting by molar mass ratio. The method is described in detail in Gutierrez et al. (2006).

2.3.4. Risk of establishment of food-borne pathogens in spent substrate

Mushroom substrate inoculated with spawn of *P. ostreatus* was prepared as described above and divided between containers, with 25 g of inoculated substrate in each container. Two food-borne pathogens were used in the experiments, *Salmonella enterica* serovar Typhimurium (CCUG-98112-08) and *Listeria monocytogenes* LM052 (Guiller et al., 2013). At the start of the experiment (d 0), the containers were inoculated with a starting concentration of 10^5 colony forming units (cfu) g^{-1} of each pathogen or left uninoculated (control). Samples were collected for monitoring pathogen concentrations on d 0, 5, 10, 20, and 30, with three separate container for each sampling point and pathogen. At each sampling, the entire contents of the container were placed in a Stomacher bag (BagFilter®, Interlab for INTERSCIENCE, France) with 225 mL of sterile 0.9% NaCl and homogenized for 120 s in a blender (Star Blender LB 400, VWR International). The homogenized sample was then plated on selective agar, and growth and establishment of the pathogens were monitored according to the procedures of the Nordic Committee on Food Analysis (*Listeria* NMKL 136, 5.Ed, 2010; *Salmonella* NMKL 71, 5. Ed., 1999).

2.4. Statistics

All experiments were set up with three replicates in each treatment. Statistical analyses were carried out using Minitab version 2018 and data were tested for significant differences ($p < 0.05$) using ANOVA and Tukey's post-hoc test and *t*-test. Data are presented as mean ± standard deviation (std).

3. Result and discussion

3.1. Mushroom production and quality

Increasing awareness of the negative effects of development based on the concept of "take, make, and dispose" is driving a societal change towards circular production systems. This calls for innovative ways to recycle nutrients from otherwise unused biomass back into food and feed production. Mushroom production on locally produced and unused plant residues is an obvious way to do this. In the present study, a waste product from faba bean production (hulls) was used as the sole substrate, without any amendments or manipulations, for production of oyster mushrooms. The fruiting bodies produced appeared typical for *P. ostreatus*, with numerous clusters in shades of grey. The time span from start to harvest was approximately one month, with pins being observed in the boxes at d 27–29 and harvest of fruiting bodies five days after emergence of the pins. Biological efficiency of the substrate, a critical production parameter describing conversion of substrate into fruiting bodies, was 109.0 ± 28.1% based on the first flush only. Oyster mushrooms are known for being productive, with BE values often exceeding 100% in well-functioning production systems (Stamets, 2000). The BE value observed in the present study would have been larger if several flushes of fruiting bodies were harvested. However, only the first flush was harvested due to increased risk for infections over time, e.g. molds, and the intended use of the remaining SMS for feed purpose.

The harvested oyster mushrooms had a moisture content of 90.8 ± 0.6% and a total protein concentration of 23.1 ± 5.0% of dwt. This value was lower than the total sum of amino acids (32.1 ± 1.9% of dwt), a discrepancy explained by total protein level being calculated based on total nitrogen levels and a conversion factor of 4.38, which is lower than the commonly used protein conversion factor of 6.25 because mushrooms are rich in non-protein nitrogen, e.g., chitin (Barros et al., 2008). However, the commonly used conversion factors of 6.25 (standard) and 4.38 (mushrooms) do not necessarily reflect the exact composition of an individual foodstuff (Mariotti et al., 2008). The amino acid composition was well in line with reported values for fruiting bodies of *P. ostreatus* (OECD, 2013). The dominant amino acids were arginine, aspartic acid, and glutamic acid (Table 1), but ornithine was also present in the

Table 1
Amino acid concentration (g 100 g^{-1}, dry weight) in faba bean hulls before inoculation with *Pleurotus ostreatus* (initial value), in the spent substrate after mushroom harvest (SMS), and in the fruiting bodies produced. Mean ± std, n = 3.

Amino acid	Content in faba bean hulls		Content in fruiting bodies	
	Initial value	SMS	*P. ostreatus*	Range of mean values**
Alanine	0.72 ± 0.04a*	1.34 ± 0.02b	1.49 ± 0.13b	0.95–2.86
Arginine	1.56 ± 0.12a	1.86 ± 0.09a	2.42 ± 0.19b	0.95–2.76
Aspartic acid	1.95 ± 0.16a	2.97 ± 0.14b	2.32 ± 0.19a	1.42–3.66
Cysteine	0.24 ± 0.02a	0.43 ± 0.02b	0.32 ± 0.02c	0.12–0.38
Glutamic acid	2.72 ± 0.21a	4.10 ± 0.26b	5.20 ± 0.31c	2.71–5.84
Glycine	0.88 ± 0.03a	1.61 ± 0.03b	1.23 ± 0.11c	0.70–1.71
Histidine	0.47 ± 0.03a	0.60 ± 0.02b	0.69 ± 0.09b	0.31–1.24
Isoleucine	0.70 ± 0.05a	1.11 ± 0.06b	0.98 ± 0.11b	0.71–1.62
Leucine	1.28 ± 0.09a	2.00 ± 0.11b	1.60 ± 0.18a	1.18–2.57
Lysine	1.15 ± 0.08a	1.11 ± 0.04a	1.51 ± 0.20b	1.10–2.29
Methionine	0.16 ± 0.01a	0.28 ± 0.02b	0.40 ± 0.05c	0.26–0.44
Phenylalanine	0.74 ± 0.06a	1.25 ± 0.06b	1.10 ± 0.11b	0.66–1.52
Proline	0.74 ± 0.06a	1.36 ± 0.02b	1.01 ± 0.11c	0.39–1.52
Serine	0.90 ± 0.06a	1.46 ± 0.06b	1.35 ± 0.11b	0.72–1.81
Threonine	0.66 ± 0.05a	1.24 ± 0.03b	1.27 ± 0.12b	0.73–1.71
Valine	0.78 ± 0.06a	1.28 ± 0.05b	1.22 ± 0.16b	0.77–2.10
Σ Amino acids	17.63 ± 0.24a	29.00 ± 1.84b	32.11 ± 1.90b	

*Values within rows followed by different letters are significantly different ($p \leq 0.05$).
**Range of mean values according to OECD (2013).

Table 2
Concentration of different elements (mg kg^{-1}, dry weight) in the oyster mushrooms produced and (in brackets) previously reported values compiled by Mleczek et al. (2018). Mean ± std, n = 3.

Element	Oyster mushroom
Al	7.4 ± 0.4 (17–85)
Ba	0.07 ± 0.01
Ca	284.2 ± 91.6 (190–1500)
Cd	0.2 ± 0.07 (0.3–5.4)
Co	0.04 ± 0.01
Cr	0.7 ± 0.2 (0.1–16.3)
Cu	24.6 ± 3.6 (19–50)
Fe	42.1 ± 3.3 (33–550)
K	16916.8 ± 8423.8 (21840-51000)
Li	0.04 ± 0.01 (0.04–0.2)
Mg	1899.2 ± 121.9 (165–2300)
Mn	4.6 ± 0.2 (5–31.5)
Mo	0.4 ± 0.1
Na	245.2 ± 53.9 (250–1440)
Ni	1.1 ± 0.5 (1.5–31.5)
P	14164.5 ± 1482.5 (618–13390)
S	2576.0 ± 185.0
Si	57.9 ± 17.1
Sr	0.3 ± 0.1 (0.1–0.6)
Ti	0.1 ± 0.03
V	0.3 ± 0.02
Zn	25.2 ± 1.0 (25–265)
Zr	0.03 ± 0.01

fruiting bodies, in a concentration of 0.6 ± 0.1% of dwt. The elemental composition of fruiting bodies of *P. ostreatus* has been compiled by Mleczek et al. (2018) and the published data was in line with the results obtained in the present study (Table 2). Mushrooms are well-known for accumulating heavy metals (Huang et at., 2015), so it is of interest to note that the cadmium content was low (Table 2).

Our results has implications for sustainable production of food as faba bean hulls proved to be well suited as substrate for production of oyster mushrooms. High productivity was observed without any need for pretreatment of the substrate apart from hydration and pasteurization. The quality of the fruiting bodies produced was comparable to that of commercial oyster mushrooms in terms of morphology, protein content, protein quality, and chemical composition. It should be pointed out that commercial mushroom producers generally use large amount of mushroom substrate, exceeding several kg of wet weight, for maximum production of fruiting bodies. Owaid et al. (2015) confirmed experimentally that higher BE values can be obtained when using substrate amounts exceeding 1 kg wet weight. In the present study, a lower amount of substrate (0.6 kg wet weight) was used, and thus it is likely that even higher BE values can be obtained in commercial production settings.

3.2. Impact of mushroom cultivation on the substrate

Fungal growth is heterotrophic, resulting in partial degradation of the substrate and release of carbon dioxide due to respiration. Following the high productivity and intense fungal growth in this study, substantial weight loss of the substrate was observed after harvest, with 48.4 ± 0.6% of initial dwt remaining. Total nitrogen concentration in the initial substrate was 3.7 ± 0.3% of dwt while in the SMS the total concentration was 5.6 ± 0.3% of dwt. Considering the weight reduction, as well as the increase in nitrogen concentration in the SMS, approximately 73.4% of the initial nitrogen amount remained in the substrate after harvest.

On comparing the proximate composition of the faba bean hulls before use and after mushroom production (in SMS), significant differences were found (Table 3). Concentrations of ash, total protein, NDF, and neutral lipids were significantly elevated after mushroom production, while WSC content showed a large decrease. As previously mentioned, *P. ostreatus* is a wood-degrading fungi (white-rot fungi) with high capability to degrade recalcitrant compounds such as lignin. The

Table 3
Impact of *Pleurotus ostreatus* production on proximate composition (g kg^{-1} dry weight (dwt)) and content of condensed tannins, vicine, and convicine (g kg^{-1} dwt) in the faba bean hull substrate. Mean ± std, n = 3.

	Initial value	SMS (after mushroom harvest)
Ash	29.5 ± 0.6a*	57.1 ± 1.6b
Total protein	207.9 ± 8.6a	346.6 ± 16.5b
Neutral detergent fiber	407.9 ± 6.8a	460.9 ± 23.3b
Water-soluble carbohydrates	25.1 ± 1.2a	1.0 ± 1.4b
Neutral lipids	13.1 ± 0.6a	31.0 ± 1.6b
Condensed tannins	18.9 ± 4.0a	9.0 ± 1.6b
Vicine	5.7 ± 0.1	nd
Convicine	1.4 ± 0.04	nd

*Values within rows followed by different letters are significantly different ($p \leq 0.05$).
**Not detected (<20 mg kg^{-1}).

Table 4
Concentrations of different elements (mg kg^{-1}, dry weight) in faba bean hulls before use (initial value) and in spent mushroom substrate after harvest (SMS). Mean ± std, n = 3.

	Initial value	SMS
Al	8.9 ± 0.6a	22.5 ± 2.4b
Ba	0.5 ± 0.02a	1.1 ± 0.07b
Ca	2172.5 ± 172.5a	6973.6 ± 624.3b
Cd	0.2 ± 0.02a	0.2 ± 0.03a
Co	0.1 ± 0.02a	0.3 ± 0.03b
Cr	0.2 ± 0.05a	0.6 ± 0.05b
Cu	10.0 ± 0.4a	17.4 ± 4.8a
Fe	39.1 ± 1.1a	88.7 ± 27.2b
K	6118.3 ± 216.3a	8024.8 ± 1114.6b
Li	0.04 ± 0.003a	0.1 ± 0.004b
Mg	1805.2 ± 84.9a	3251.7 ± 134.9b
Mn	4.9 ± 0.3a	18.6 ± 0.3b
Mo	1.1 ± 0.01a	2.1 ± 0.01b
Na	148.4 ± 4.0a	312.1 ± 14.2b
Ni	4.6 ± 1.8a	4.6 ± 0.8a
P	2559.0 ± 99.5a	3237.2 ± 584.3a
S	978.3 ± 32.1a	2813.4 ± 67.8b
Si	37.4 ± 5.6a	661.1 ± 30.0b
Sr	1.7 ± 0.1a	8.6 ± 0.8b
Ti	0.1 ± 0.02a	0.3 ± 0.06b
V	0.3 ± 0.01a	0.4 ± 0.04b
Zn	11.4 ± 0.2a	17.9 ± 0.8b
Zr	0.01 ± 0.004a	0.1 ± 0.03b

*Values within rows followed by different letters are significantly different ($p \leq 0.05$).

significant increase in NDF concentration, representing the lignocellulosic fraction, observed in the SMS can be explained by preferential use of the easy accessible carbohydrates (WSC) by the fungus and mass reduction of the substrate. In line with the significant increase in ash concentration after mushroom harvest, the concentrations of all elements analyzed, except for cadmium, copper, nickel, and phosphorus, were significantly increased in the SMS compared with the raw hulls (Table 4). Total protein level differed in the substrate before and after mushroom harvest. Protein quality also differed, with significant increases in all amino acids except arginine, histidine, and lysine in the substrate after mushroom harvest (Table 1). Ornithine, which was seen in the fruiting bodies, was also present in the substrate after mushroom harvest, but in very low concentrations (0.04 ± 0.01% of dwt). This amino acid was not detected in faba bean hulls before inoculation with *P. ostreatus* and its detection clearly indicated that the SMS was partly composed of fungal mycelium.

Lactic acid fermentation of faba bean flour has previously been demonstrated to decrease the levels of anti-nutritional factors such as vicine and convicine (Rizzello et al., 2016). In the present study, the initial concentration of vicine was 5.7 ± 0.1 g kg^{-1} dwt and that of convicine was 1.4 ± 0.04, while the concentration of both compounds

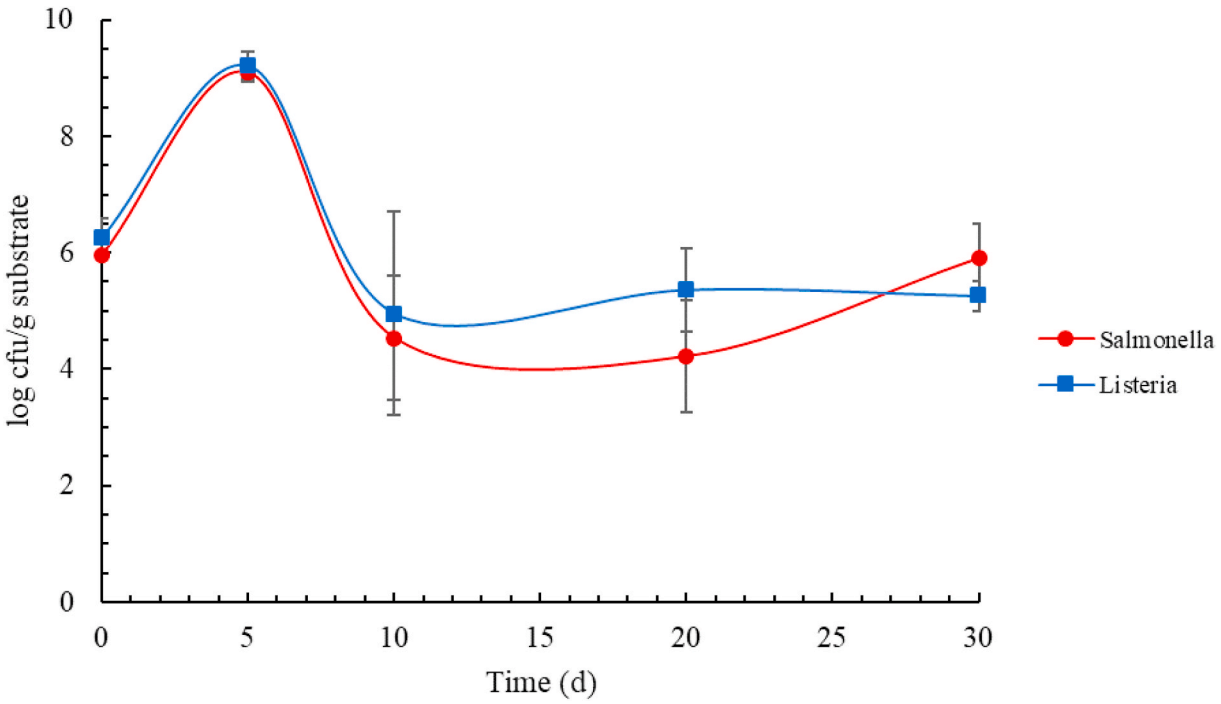

Fig. 1. Change in concentration (log cfu g^{-1}) of the food-borne pathogens *Salmonella enterica* serovar Typhimurium and *Listeria monocytogenes* in the mushroom substrate over time after initial inoculation at 6 log cfu g^{-1} substrate. Mean ± std, n = 3.

was below the detection limit (<20 mg kg^{-1}) in the SMS (Table 3). Thus, production of *P. ostreatus* was highly efficient in reducing the levels of these compounds. This is less well-studied in the literature, where the focus is mainly on fruiting body production rather than SMS quality. In one of the few studies performed to date, production of *P. ostreatus* on two different bean varieties (*Phaseolus vulgaris*; black beans and kidney beans) resulted in significantly decreased content of condensed tannins (Espinosa-Páez et al. (2017). Condensed tannins are mainly located in the hulls of faba beans (Sinha and Kumar, 2018; Helsper et al., 1993). In the study by Espinosa-Páez et al. (2017) the two bean varieties had an initial condensed tannin concentration below 1 g kg^{-1} dwt, while a high initial concentration of 18.9 ± 4.0 g kg^{-1} dwt was observed in the hulls used in the present study. This initial concentration was significantly reduced in the SMS, to approximately 50% of the initial value (Table 3). *Pleurotus ostreatus* produces several degrading enzymes, but with a clear dominance of laccases when grown on lignocellulosic substrate (Fernández-Fueyo et al., 2016), as in the present study. In previous analysis of the laccase activity of the *P. ostreatus* strain used in the present study, especially high levels were observed immediately before fruiting body induction (Hultberg et al., 2020). Thus, the concentration of anti-nutritional factors in legume residues can most probably be reduced by their use as substrate for *P. ostreatus* growth to mycelium stage, without the technically challenging fructification phase.

3.3. Use of SMS as a feed ingredient in pig diet

As previously pointed out, SMS has potential as a feedstuff for animals and thereby implications for lowering dependence on imported protein feeds in the European Union. The main aspect to consider in this regard is that SMS contains chitin as a major structural component, due to the presence of fungal mycelium. Clear differences in expression of chitinase, the enzyme capable of degrading chitin, between omnivores, herbivores, and carnivores have been reported, with omnivores such as pigs and poultry having the highest capability for chitin degradation (Tabata et al., 2018). Another important aspect is the hygienic quality of the SMS. Mushroom production is performed under humid aerobic conditions at room temperature with near-neutral pH, and thus establishment of food-borne pathogens in the substrate is not hindered by environmental conditions in the same way as for fermentation processes such as lactic acid fermentation. To our knowledge, no information is available regarding the risk of establishment of food-borne pathogens in SMS, and therefore challenge tests on common pathogens were performed in the present study (Fig. 1). An initial increase in the concentration of both test pathogens from 10^5 cfu g^{-1} (d 0) to 10^9 cfu g^{-1} (d 5) was observed. This was probably due to fungal degradation of the substrate, accompanied by increased availability of nutrients to the microflora present. Over time, decreases in concentrations of the inoculated *Salmonella* and *Listeria* strains were observed, indicating that the environment was not favorable for their growth. However, the results also clearly demonstrated that the concentrations of these food-borne pathogens did not decline to sanitary levels. It is important to point out that the substrate undergoes initial pasteurization and cannot be considered a high-risk source for transfer of pathogens into the food chain. Still, high hygiene standards are needed to avoid contaminating the substrate upon inoculation of spawn or during the rest of the growth cycle, to ensure consistent hygiene quality. The pH in the initial substrate was 6.5 and in the SMS it had decreased to 5.3 ± 0.1. From a hygiene perspective this decrease is advantageous as a previous study have shown low prevalence of *Salmonella* on pig farms using automated liquid feeding of by-products, which was explained by acids in the by-products and thereby a low pH of the feed (Van der Wolf et al., 1999).

The concentrations of selected nutrients in the SMS produced in the present study were compared with those in distillers dried grains with solubles (DDGS) (SLU, 2011), a commonly used protein feed ingredient. They were also compared against the nutrient requirements of growing barrows (NRC, 2012). The concentrations of total protein, lysine, threonine, and valine were similar to those in DDGS and well above the requirement of growing barrows (Table 5). These findings indicate that SMS has potential to be a valuable protein source in pig diets. The high NDF content in SMS resulted in a lower net energy value compared with DDGS and the growing pig requirement, but this could be overcome by including SMS as one of several ingredients in a diet formulation. The cadmium level was below the maximum permissible level in vegetable feed ingredients (1 ppm (μg g^{-1}); EC 2013) and, although most elements

Table 5
Proximate composition, concentrations of selected amino acids (g kg^{-1} dry weight (dwt)) and calculated net energy content (MJ kg^{-1} dwt) (EvaPig® Version 1.4.0.1) of distillers dried grains with solubles (DDGS) (SLU, 2011) and of spent mushroom substrate after harvest (SMS), and the corresponding requirements of growing pigs (barrows weighing 75–100 kg) according to NRC (2012).

	DDGS	SMS	Pig requirement
Ash	44	57.1	.
Total protein	340	346	129
Neutral detergent fiber	354	461	..
Neutral lipids	66.0	31.0	
Water-soluble carbohydrates	14.0	1.0	
Lysine	10.6	11.1	8.88
Methionine	6.3	2.8	2.5
Cystine	8.1	4.30	2.8
Threonine	11.7	12.4	6.0
Valine	12.1	12.8	6.1
Net energy supplied to barrows	8.1	7.1	11.6

analyzed were accumulated in the SMS, the levels were below those considered to pose a risk to pig health (NRC, 2012). Condensed tannins are well known to decrease the protein digestibility of pig diets, but the level of condensed tannins in commonly used colored-flower faba bean cultivars is not considered to be a major issue in growing pig diets (Ivarsson and Neil, 2018). The level of condensed tannins in SMS (9.02 g kg^{-1} dwt) was slightly higher than that reported by Ivarsson and Neil (2018) for whole beans of the cultivar 'Alexia' (7.68 g kg^{-1} dwt). A maximum tolerance level of tannins in pig diets has not been established but, apart from lower protein digestibility, tannins might decrease feed intake due to a bitter taste (Jansman, 1993). To fully explore the potential of SMS as a feed ingredient, future research including animal studies to determining its digestibility and palatability are necessary.

4. Conclusions and prospects

This study demonstrates the potential of using edible wood-degrading mushrooms for valorization of agro-waste, achieving complete circular use of a waste for production of food and feed. Faba bean hulls, agro-waste with high content of anti-nutritional factors, were used as the sole mushroom substrate. No amendments or manipulations besides pasteurization were applied to the faba bean hulls, which proved to be well suited for production of oyster mushrooms. Concentrations of the anti-nutritional factors were significantly reduced in the remaining substrate after mushroom cultivation. In parallel with this reduction, the protein content was significantly increased in the remaining substrate. Thus, it can be concluded that mushroom cultivation improved the feed properties of the hulls. An important future research direction is animal studies to determining the digestibility and palatability of the remaining substrate. Furthermore, integrating the mushroom production with the feed production will add a new dimension to agricultural production. The need for controlled high humidity during mushroom production imposes different demands on climate control and equipment than in plant production. From an applied perspective teaching and information efforts are crucial as new skills and techniques need to be adopted at farm level.

CRediT authorship contribution statement

E. Ivarsson: Formal analysis, Resources, Data curation, Writing – original draftt, preparation. **M. Grudén:** Methodology, Formal analysis, Writing – review & editing. **J. Södergren:** Methodology, Formal analysis, Writing – review & editing. **M. Hultberg:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, preparation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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This work did not involve any studies with human participants or animals performed by any of the authors.

Informed consent

All authors have the authority to publish this material and have agreed to submit it to Journal of Cleaner Production.

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