



REVIEW

From Spent Mushroom Substrate to Biofeed: Source Heterogeneity, Dual Cell-Wall Barriers, and Targeted Bioprocessing

Hongyu Chen^{1,†}, Ying Wang^{1,†}, Bing Li¹, Youran Shao¹, Gen Zou^{1,*}

¹ National Engineering Research Center of Edible Fungi, Key Laboratory of Applied Mycological Resources and Utilization, Ministry of Agriculture and Rural Affairs, Institute of Edible Fungi, Shanghai Academy of Agricultural Sciences, Shanghai 201403, China.

† These authors contributed equally.

*Corresponding Author: Gen Zou, E-mail: zougen@sibs.ac.cn

Abstract

Background: Industrial mushroom cultivation generates large, concentrated quantities of spent mushroom substrate (SMS), but SMS is not a uniform feed ingredient. Mushroom species, the original substrate, the cultivation cycle, and storage history all influence its nutrient content, safety, digestibility, and sensory properties. **Purpose:** This review examines SMS as a biofeed resource and asks how different sources can be made safer, more digestible, and more acceptable to animals. **Methods:** This critical narrative review integrates source-characterisation studies, mechanistic evidence, and animal-feeding trials, giving greater weight to studies that report SMS source, treatment, and dietary inclusion. **Results:** SMS retains lignocellulose, fungal biomass, protein, minerals, and bioactive metabolites. Its feeding value is limited by two distinct structural materials: residual plant cell walls and newly formed fungal walls containing chitin, beta-glucans, and mannoproteins. Mushroom volatiles, bitter compounds, high water-holding capacity, ash, and storage spoilage may further reduce intake. Feeding studies support moderate roughage replacement in ruminants and rabbits and low-dose functional use in pigs, poultry, and tilapia, but the response depends strongly on source, treatment, dose, and diet formulation. **Conclusions:** A more practical approach is to identify what limits each batch and then decide whether it can be used directly, preserved, treated with enzymes, fermented with selected cultures, or kept out of feed. A provisional four-grade research hypothesis is proposed to organise feed suitability and treatment requirements; it is intended for future validation rather than as an established feed standard. The practical question is which material can be used, for which animal, at what inclusion level, and after what treatment.

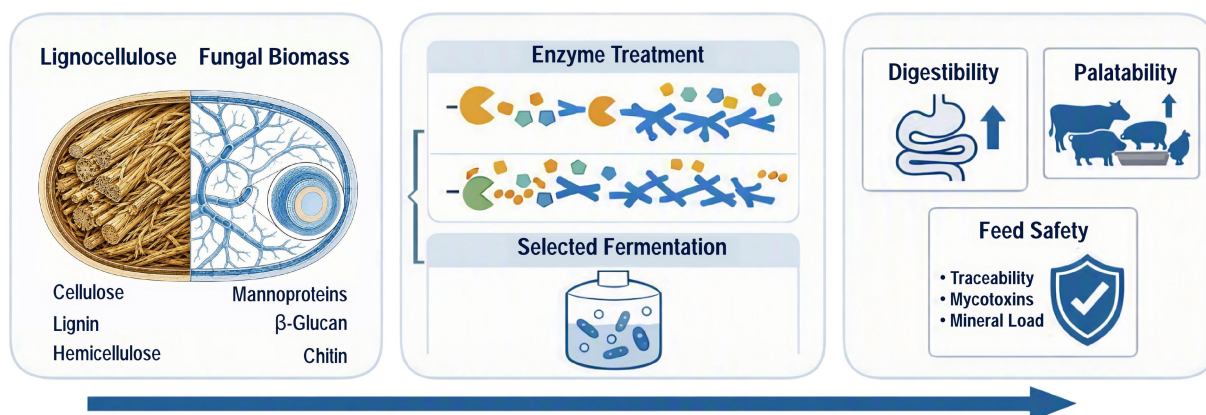
Keywords

Spent mushroom substrate; Biofeed; Chitin; Beta-glucan; Palatability; Dietary safety

Citation: Hongyu Chen, Ying Wang, Bing Li, *et al.* From Spent Mushroom Substrate to Biofeed: Source Heterogeneity, Dual Cell-Wall Barriers, and Targeted Bioprocessing. *Biofeed Science and Technology*. 2026, 01 (2): 1-17. doi: 10.66729/bst202608004.



Graphic Abstract



NOTE: SMS: spent mushroom substrate. Copyright: ©2026 The Authors. Graphic abstract assembled and edited by the authors using Microsoft PowerPoint (version 16.0); some visual elements were generated with Microsoft Copilot and further refined by the authors. The graphic abstract is not reproduced or adapted from any previously published source.

Highlights

- The review treats spent mushroom substrate (SMS) as a family of source-dependent materials rather than as a single feed ingredient.
- It identifies residual plant cell walls and newly formed fungal cell walls as two distinct structural barriers that limit nutrient accessibility and require different enzyme activities.
- It compares feeding evidence across ruminants, pigs, poultry, rabbits, aquatic animals, and indirect conversion systems, showing that feed value depends on source, treatment, dose, diet, and animal species.
- It argues that enzyme treatment and selected fermentation should be chosen according to the actual limitations of each batch and proposes a provisional four-grade scheme to organise feed suitability and treatment needs.

1. Introduction

Every kilogram of cultivated mushrooms leaves several kilograms of residual substrate, and animal feed is one of the few uses capable of accommodating material on this scale. Mushroom cultivation is unusual because a living fungus transforms a lignocellulosic substrate before the main product is harvested. The remaining material therefore contains plant residues, extracellular fungal metabolites, and living or senescent mycelium. The solid cultivation material remaining after mushroom harvest is commonly termed spent mushroom substrate (SMS). “Spent mushroom compost” is used mainly for compost-based cultivation systems such as *Agaricus*, whereas “mushroom waste” is a broader term that may also include discarded fruiting bodies, trimming residues, and processing wastes. These terms are therefore not interchangeable because they refer to materials of different origin and composition. Here, SMS refers specifically to the solid cultivation substrate remaining after a normal fruiting cycle has been completed.

The amount generated warrants serious consideration as a feed resource, although global totals should be treated as approximate. Global mushroom consumption was projected to reach 20.84 million tonnes in 2026, and bag-based cultivation commonly produces about 5 kg of wet SMS for each kilogram of fresh mushrooms^[1]. On that basis, annual production would exceed 100 million tonnes of wet material. Broader production statistics give still higher estimates^[2], but differences in cultivation method, moisture basis, and statistical coverage make direct

comparison difficult. The pattern of supply is more informative than any single total: SMS is produced continuously, at known sites, and often in industrial parks where several mushrooms are cultivated. It is a concentrated agro-industrial co-product rather than a scattered field residue.

SMS is not nutritionally exhausted. *Hypsizygus marmoreus* SMS retains substantial cellulose, hemicellulose, crude fibre, and amino acids and can support further fungal cultivation^[3]. In a comparison of seven industrial SMS sources, *H. marmoreus* SMS contained 36.75% total sugars, 28.20% crude fibre, and 10.99% crude protein. It also replaced silkworm pupae powder in *Cordyceps militaris* cultivation and increased fruiting-body biomass by about 35%^[4]. The spent material is therefore far from inert.

SMS can be used for second crops, feed, fertiliser, methane, bioethanol, enzymes, polysaccharides, materials, and bioremediation, although most of these applications remain at pilot scale^[4]. Recent studies of non-grain feed resources have likewise emphasised that potential nutritional or functional benefits need to be considered together with source-specific constraints on feed use^[5,6]. Abundance alone, however, does not make SMS suitable for feed. It may contain excessive moisture, ash, minerals, lime or gypsum, cottonseed-hull-associated risks, storage moulds, poorly digested polymers, and strong fungal aromas or tastes. It also contains two different structural materials: residual plant cell walls and fungal cell walls formed during cultivation. This helps explain why proximate composition can overstate the nutrients available to the animal and why different SMS sources require different forms of processing.

Previous reviews have described the many agricultural, material, energy, and nutritional uses of SMS^[7–9]. This review focuses more narrowly on feed and makes three contributions. First, it treats SMS not as one ingredient but as a family of materials whose composition, safety, and feed value are set by mushroom species, original substrate, cultivation cycle, and post-harvest history, and it proposes a source classification for reporting and comparison. Second, it identifies a dual cell-wall barrier characteristic of SMS: residual plant walls and newly formed fungal walls coexist in the same material, differ chemically, require different enzymes, and are obscured when combined in a single fibre value. Third, it relates the

main limitations of each batch to suitable treatments and animal testing, and uses a provisional four-grade scheme to organise the evidence. This is a critical narrative review rather than a systematic review. Greater weight is given to studies that report mushroom species, original substrate, treatment, and dietary inclusion, because findings cannot otherwise be assigned to a clearly described material, and evidence from purified fungal walls, mushroom powders, or mycelial biomass is used to explain mechanisms rather than as a substitute for feeding studies on SMS itself. **Figure. 1** shows how source and safety considerations shape the choice between feed and non-feed uses.

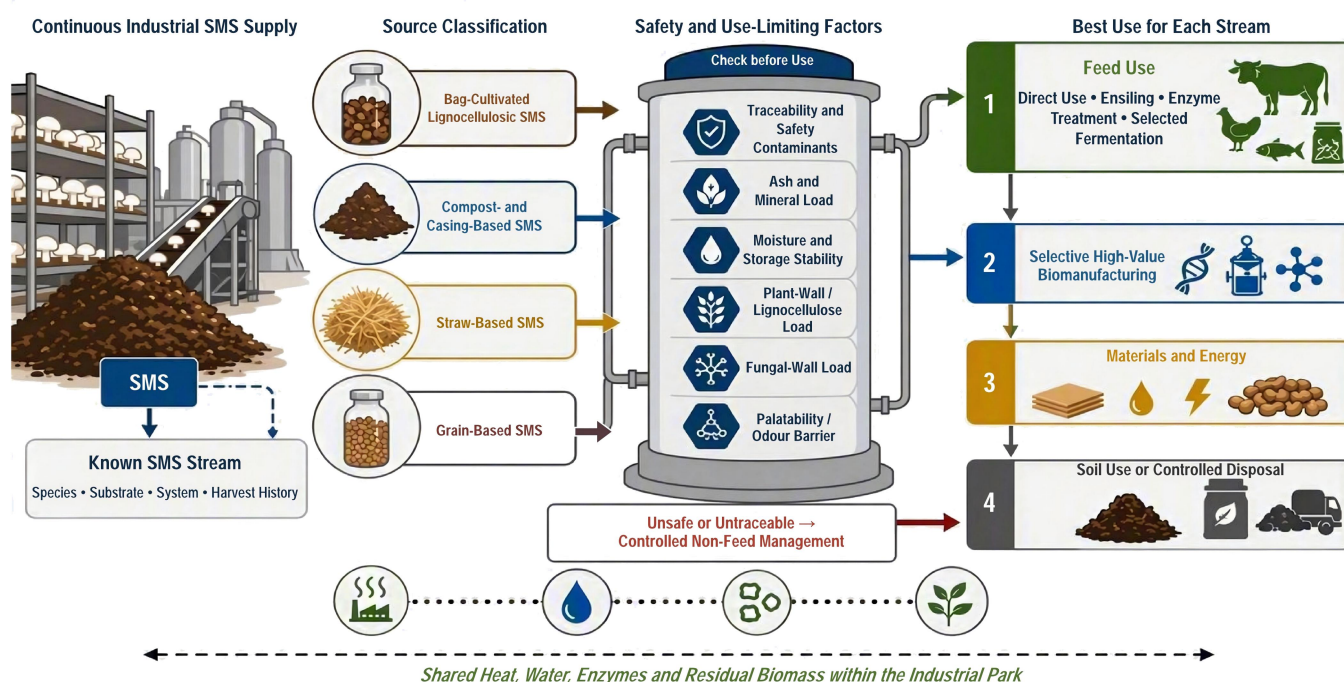


Figure. 1 Source screening and tiered use of spent mushroom substrate. SMS should first be described by mushroom species, original substrate, cultivation system, and harvest and storage history. Safety, moisture, mineral load, residual plant material, fungal biomass, and palatability determine whether a batch can be used directly, ensiled, treated with enzymes, or fermented with selected cultures. Material that is not practical for feed may be better used for selective biomanufacturing, materials, energy, soil amendment, or controlled disposal. The lower line shows possible sharing of heat, water, enzymes, and residual biomass within an industrial park; it does not imply that rejected material returns to feed. **NOTE:** SMS: spent mushroom substrate. Copyright: ©2026 The Authors. Figure assembled and edited by the authors using Microsoft PowerPoint (version 16.0); some visual elements were generated with Microsoft Copilot and further refined by the authors. The figure is not reproduced or adapted from any previously published source.

2. SMS is a Family of Feedstocks, Not a Single Feed Ingredient

2.1 Source Terminology and Production Context

Reports on SMS should describe its source. At a minimum, they should identify the mushroom species, original substrate formulation, cultivation method, number or duration of harvest flushes, presence of casing, and post-harvest handling. The starting recipe cannot replace analysis of the spent material. In a direct comparison of oyster-mushroom SMS with the sterilised substrate from which it was produced, cultivation changed moisture, ash, pH, the carbon-to-nitrogen ratio, and several macro- and micronutrients^[10]. The fresh substrate and the material leaving the farm are therefore chemically different, and studies cannot be compared meaningfully when these details are missing.

For feed development, SMS can be grouped into four broad source types. Bag-cultivated lignocellulosic SMS from *Lentinula*, *Pleurotus*, *Flammulina*, *Hypsizygus*, and related mushrooms

contains partially transformed sawdust, corncob, cottonseed hulls, straw, or bran together with variable amounts of fungal biomass. Even shiitake and oyster-mushroom SMS differ in recoverable phenolics, carbohydrates, and beta-glucans^[11]. Compost- and casing-based SMS from *Agaricus* production may contain manure-derived nitrogen, gypsum, lime, and casing soil, so ash, calcium, and salinity require particular attention; electrical conductivity of 18–23 dS/m has been reported for spent and recomposted *Agaricus* substrates^[12]. Straw-based SMS from *Volvariella* and similar systems is best considered first as a fibrous roughage. Grain-based medicinal-mushroom SMS, including *C. militaris* residues, may contain more fungal biomass and residual protein, together with a higher chitin load and carryover of bioactive metabolites. Liquid-fermentation biomass, mushroom-processing residues, and materials discarded before a fruiting cycle is completed are not included in the definition used here.

The source matters in practice. Seven industrial SMS types produced responses ranging from growth promotion to inhibition when used in the same *C. militaris* cultivation system^[1]. Differences

in soluble metabolites, organic acids, nutrients, and lignocellulosic fractions were associated with these responses. Bioactive metabolites carried over in *H. marmoreus* SMS were also shown to stimulate *Pleurotus ostreatus* growth^[13]. These experiments are not animal-feeding trials, but they show that material sold under the same broad name can behave very differently even at the same nominal dose.

Direct quantitative comparison among the four source groups remains difficult, because most studies do not report crude protein, ash, chitin, and beta-glucan on a common dry-matter basis using comparable analytical methods. Representative values nevertheless illustrate the scale of variation. In one comparison of seven industrial SMS sources, *H. marmoreus* SMS contained 10.99% crude protein and 28.20% crude fibre^[1], whereas the *P. ostreatus* SMS used in a Hu-sheep feeding study contained 29.41% ash^[30]. Spent and recomposted *Agaricus* substrates have also shown electrical conductivity of 18–23 dS/m^[12], reflecting the distinctive mineral load of compost-based materials. Comparable cross-source ranges for chitin and beta-glucan are not yet available because these fungal-wall components are rarely quantified in animal-feeding studies at all. This lack of harmonised compositional data is itself an important barrier to source comparison and standardisation.

2.2 Composition is Created by Both the Original Substrate and the Cultivated Fungus

SMS composition reflects both what the mushroom consumed and what it produced. During cultivation, the fungus uses soluble sugars and nitrogen, modifies lignin, cellulose, and hemicellulose, secretes extracellular enzymes, and adds new fungal biomass. An apparent increase in crude protein or ash can arise from fungal growth, selective loss of carbohydrate, or concentration after dry-matter loss. This distinction matters. When wheat straw, miscanthus, and wood chips were treated with *Ganoderma lucidum*, *Lentinula edodes*, *Pleurotus eryngii*, or *P. ostreatus*, cellulose concentration increased in every substrate, yet the absolute amount of cellulose had declined^[14]. A higher percentage of crude protein or cellulose therefore does not

necessarily mean that more digestible nutrients remain. Composition should be reported together with dry-matter recovery.

Mushroom cultivation can loosen lignocellulosic structure and, in some cases, improve the use of fibrous residues by ruminants. In a screen of 32 strains of *Ceriporiopsis subvermispora*, *P. eryngii*, and *L. edodes* on wheat straw, 17 strains increased in vitro gas production, and the best *C. subvermispora* strains reached 297 mL/g rather than 228 mL/g organic matter^[15]. The benefit, however, varies widely. Across wheat cultivars and maturity stages, lignin degradation ranged from 47% to 93.50% and the increase in gas production from 15.30% to 47.60%^[16]. Under farm conditions, three fungi including *P. ostreatus* and *Volvariella volvacea* instead reduced in vitro degradability over 42 days, with neutral detergent fibre digestibility falling by as much as 30% of the original value^[17]. The fungus consumes accessible carbohydrate as well as lignin, so the remaining material may become enriched in recalcitrant lignin or crystalline cellulose. Absolute recovery of degradable organic matter is therefore the more informative measure, since a favourable shift in percentage composition can accompany a net loss^[18]. The outcome depends on fungal species and strain, substrate, and harvest intensity^[7].

Cultivation also adds compounds that were not present in the original plant material, including chitin, beta-1,3/1,6-glucans, mannoproteins, enzymes, organic acids, transformed phenolics, and volatile compounds. Metabolomic analysis of oyster-mushroom SMS showed both loss and formation: many low-molecular-weight compounds, including polyphenols, declined, whereas others such as cerebroside B and ganoderenic acid D became more abundant^[19]. SMS can also retain ligninolytic enzymes, although their activity falls during storage unless the material is preserved^[20]. Some fungal products may support gut health at low doses, while others increase viscosity, water holding, bitterness, or the amount of material reaching hindgut fermentation. Feed evaluation therefore needs to consider both the residual plant material and the fungal biomass formed during cultivation. The main source groups and their feed-relevant features are summarised in **Table 1**.

Table 1. Main SMS source groups and their relevance to feed use

Source Group	Typical Material	Possible Feed Value	Main Concerns	First Questions to Ask
Bag-cultivated lignocellulosic SMS	Sawdust, corn cob, cottonseed hulls, straw, wheat bran; variable mycelial biomass	Partly delignified fibre, fungal protein, beta-glucans, residual enzymes	High fibre, two cell-wall types, cottonseed-associated factors, variable ash	Measure plant- and fungal-wall fractions and check source-specific risks
Compost- and casing-based SMS	Straw/manure compost, gypsum or lime, and possible casing material	Fermentable fibre and minerals	High ash, calcium, salts, soil carryover, variable compost maturity	Remove casing where practical; check ash, salts, minerals, and contaminants
Straw-based SMS	Rice or wheat straw and cultivation supplements	Structured fibre with roughage potential for ruminants and rabbits	Low energy density, silica, seasonal variation, storage spoilage	Preserve promptly, then test degradability and intake

Table 1. Main SMS source groups and their relevance to feed use (continued)

Source Group	Typical Material	Possible Feed Value	Main Concerns	First Questions to Ask
Grain-based medicinal-mushroom SMS	Rice, wheat, corn, or mixed grain fruiting substrates	Potentially higher residual protein and fungal biomass; functional metabolites	Variable chitin-glucan content, carryover of bioactive metabolites, oxidation and storage risks	Check fungal-wall load, storage stability, safety, and dose-related effects

NOTE: SMS: spent mushroom substrate.

3. Structural, Nutritional, and Sensory Constraints on SMS Feed Utilisation

3.1 The Dual Cell-Wall Barrier

The defining structural feature of SMS is the presence of two different cell-wall materials. Residual plant tissue contains cellulose microfibrils, hemicellulose, lignin, and lignin-carbohydrate complexes. Fungal biomass contains walls built mainly from chitin, beta-1,3-glucan, beta-1,6-glucan, mannoproteins, and associated glycoproteins^[21,22]. Fungal walls are continually remodelled during growth and stress, so their resistance to digestion cannot be predicted from composition alone^[23]. Plant and fungal walls differ chemically and require different enzymes; combining them in a single crude-fibre value hides this distinction.

The fungal wall forms a physical barrier around intracellular protein. Studies of mycoprotein show that intact hyphae limit protein release during gastrointestinal digestion^[24]. In *C. militaris*, promoter editing increased the expression of an endo-N-acetyl-beta-D-glucosaminidase involved in outer-wall mannoprotein remodelling, producing a strain with less chitin and crude polysaccharide, lower viscosity and fewer residual solids during simulated digestion, and greater amino-acid release^[25]. These findings show why crude protein alone is a poor guide to the nutritional value of fungal biomass: nitrogen-containing chitin can inflate crude-protein estimates, while intact fungal walls can restrict the release and digestion of intracellular protein.

Enzyme treatment of *P. eryngii* likewise increased protein extraction, amino-acid release, and the formation of short peptides. The most effective preparation differed between mycelium and fruiting body, and a higher dose was not always better^[26]. This difference is directly relevant to SMS. A material rich in mycelium will not necessarily respond to the same enzyme mixture as one dominated by plant residue or fruiting-body fragments.

3.2 Beta-Glucans and Chitin: Functional Compounds at Low Dose, Structural Burdens at High Load

Fungal beta-glucans and chitin are not simply antinutritional factors. Purified or standardised beta-glucans can modulate immunity, and chitin-derived oligosaccharides may also be biologically active. Their effects depend on molecular weight, branching, solubility, and conformation^[27]. In SMS, however, these polymers occur mainly in insoluble, cross-linked walls whose structure and accessibility are usually unknown. At high inclusion, they may dilute dietary energy, retain water, increase digesta bulk, restrict access to protein, and place more fermentable material in the hindgut.

Tolerance also differs among animals. Ruminants can use complex fibre through rumen fermentation, whereas young pigs, poultry, and aquatic animals are less able to handle large amounts of plant and fungal structural carbohydrate. The same material may therefore be a useful fibre source in one species and a diluent in another.

The nutritional effect of these wall polymers is therefore dose- and host-dependent rather than intrinsically beneficial or detrimental. Residual lignocellulose primarily acts as a structural energy constraint, although animals with extensive microbial fermentation can recover part of this energy. Fungal chitin-glucan walls have a second effect: they restrict access to intracellular protein, while accessible beta-glucans or chitin-derived products may exert functional effects at relatively low exposure. As the amount of intact wall material increases, however, nutrient dilution, water retention, digesta bulk, and transfer of poorly digested substrate to distal fermentation become increasingly important. The same SMS can therefore shift from a low-dose functional ingredient to a structural burden as inclusion increases, depending on wall accessibility and the digestive physiology of the animal.

3.3 Protein Concentration is Not Protein Value

Kjeldahl nitrogen can overestimate fungal protein because chitin also contains nitrogen^[25]. Fermentation can create a second source of overestimation: microorganisms consume carbohydrate and release carbon dioxide, so crude protein may rise as a percentage even when the amount of recoverable amino acid changes little. Adding yeast also adds microbial nitrogen together with another beta-glucan-containing, mannan-containing, and chitin-containing wall.

Protein value is better judged from soluble protein, amino-acid composition, in vitro digestibility, peptide-size distribution, and, where possible, ileal or total-tract digestibility in the intended animal. Wall opening and proteolysis need separate evaluation. A treatment may generate peptides from extracellular protein while leaving most intracellular protein trapped. Conversely, wall opening may increase protein bioaccessibility without ensuring digestibility, because the released protein must still be susceptible to gastrointestinal proteolysis and converted into absorbable peptides and amino acids.

3.4 Mushroom Aroma, Bitterness, and Palatability

Mushrooms have characteristic volatile profiles dominated by eight-carbon (C8) compounds such as 1-octen-3-ol, together with 1-octen-3-one, 3-octanone, and related alcohols and ketones^[28]. SMS may also carry odours from the original substrate, phenolic notes, organic acids, oxidation products, and compounds formed during poor storage. Its smell and taste therefore reflect both the cultivated fungus and the substrate on which it grew.

Direct evidence that individual mushroom volatiles cause feed refusal is still limited, and it would be premature to attribute poor intake to 1-octen-3-ol alone. Feeding studies nevertheless show that sensory and physical acceptance deserves attention. Intake and digestibility fell at 30% inclusion in sheep^[29], feed efficiency worsened at 20% in Hu sheep^[30], and finishing calves used the same *Agaricus* residue more successfully as pellets than as mash^[31]. None of these studies measured volatiles or included preference tests. Fibre dilution, mineral load, texture, and selective feeding may all

have contributed alongside flavour. Voluntary intake, sorting, texture, and water-holding capacity should therefore be measured with chemical composition, and strong mushroom, musty, woody, bitter, or acidic notes should be quantified rather than assumed to be harmless or decisive.

A *C. militaris* editing study offers a useful link between wall structure and taste. Wall remodelling was accompanied by less

bitterness and greater umami intensity in fruiting bodies^[25]. This does not show that the same change will improve animal intake, but it suggests that wall turnover, amino-acid release, and flavour can change together. Flavour should therefore be measured after processing rather than simply masked with generic flavouring agents. **Figure 2** summarises the structural, sensory, and physical constraints considered in this review.

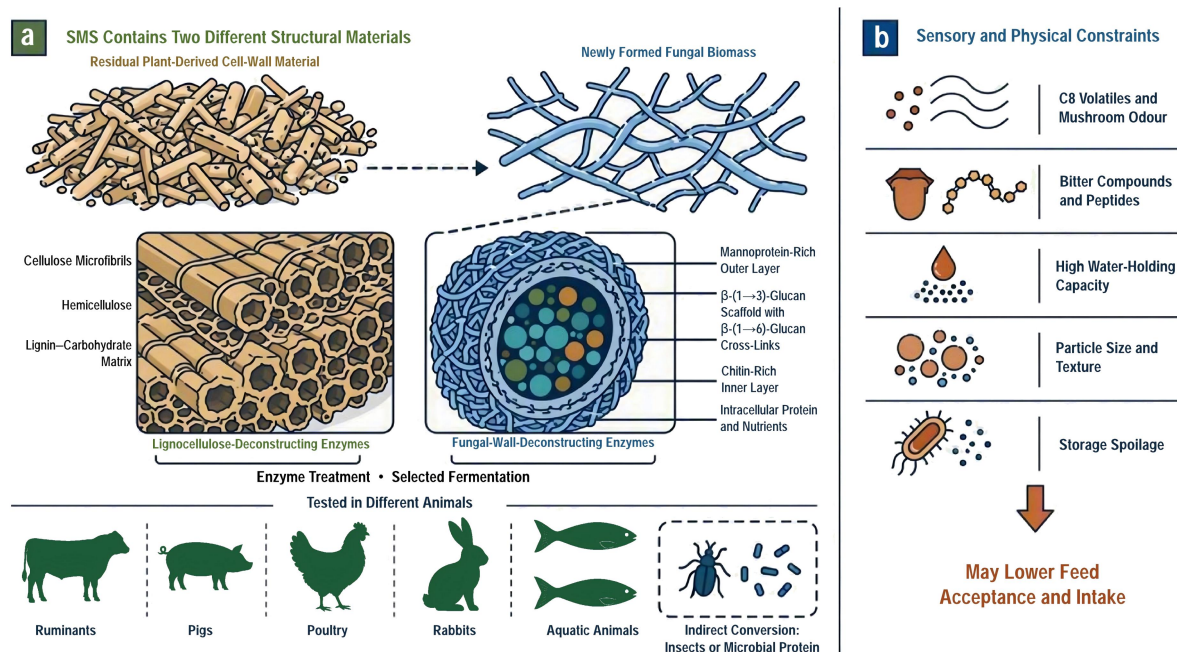


Figure 2 Structural, sensory, and physical constraints on the feed use of spent mushroom substrate. SMS contains residual plant cell-wall material and newly formed fungal biomass, which are separate structural materials and require different enzyme activities. Enzyme treatment and selected fermentation may be used separately or together, depending on the material. Mushroom volatiles, bitter compounds and peptides, high water-holding capacity, particle size, texture, and storage spoilage may reduce feed acceptance. Their importance varies among animals and must be tested directly. Insects and microbial protein are shown as possible indirect uses. **NOTE:** SMS: spent mushroom substrate; C8: eight-carbon compounds. Copyright: ©2026 The Authors. Figure assembled and edited by the authors using Microsoft PowerPoint (version 16.0); some visual elements were generated with Microsoft Copilot and further refined by the authors. The figure is not reproduced or adapted from any previously published source.

4. Evidence from Animal Feeding: Benefits Depend on Source and Inclusion Level

Animal responses to SMS can therefore be interpreted as the combined outcome of source-dependent wall composition, the extent to which processing alters wall accessibility, dietary inclusion level, and the digestive physiology of the recipient species. Ruminants have the greatest capacity to recover energy from structural carbohydrate, because plant residues are exposed to extensive microbial fermentation before the small intestine, although lignification and poorly accessible fungal-wall material can still limit their use. Rabbits also obtain substantial energy from fibre through caecal fermentation, but this occurs after small-intestinal digestion and therefore does not fully compensate for low dietary energy density or poor feed acceptance. Pigs and poultry depend more strongly on nutrient release before hindgut or caecal fermentation and are therefore more sensitive to nutrient dilution, wall-entrapped protein, and, for soluble polysaccharides, increased digesta viscosity, whereas in fish the ability to use structural carbohydrate is species- and dose-dependent. The same SMS can therefore act mainly as a roughage source in ruminants and rabbits but is more often used at low functional doses in pigs, poultry, and fish.

4.1 Ruminants

The studies below are not directly comparable. Inclusion is reported on fresh-weight, dry-matter, complete-diet, concentrate, or forage bases; trials range from short digestibility studies to full fattening periods; control diets differ in energy and protein; and many papers identify SMS only by mushroom species. Inclusion levels are therefore reported as published and interpreted in the context of the material description provided by the authors. Comparisons among animal species should be read as qualitative.

Ruminants are the most obvious candidates because rumen microorganisms ferment structural carbohydrates and SMS can replace part of the roughage. Microbially fermented SMS supported growth in Hanwoo steers^[32], and ensiling can improve preservation and alter fibre degradability without making SMS equal to conventional forage^[33,34]. A recent study separated these effects: in *Flammulina velutipes* and *P. eryngii* SMS silages, lactic-acid bacteria and cellulase improved fermentation quality and aerobic stability, whereas cellulase increased *in vitro* neutral detergent fibre and acid detergent fibre digestibility^[35]. Preservation and fibre breakdown are therefore different benefits. Practical limits are also clear: sheep tolerated casing-removed *Agaricus bisporus* compost at up to 20% of diet dry matter, whereas 30% reduced intake and digestibility^[29], and in finishing

Holstein calves 15% of the same residue performed better as pellets than as mash^[31]. *F. velutipes* SMS silage has been associated with fewer rumen protozoa and lower enteric methane in Holstein steers^[36], although it was ensiled with whole-crop corn and sometimes urea.

The amount included remains decisive. In Hu sheep, *P. ostreatus* SMS replaced whole-plant corn silage at 5%–20%. Intake and daily gain were unchanged, but 20% worsened the feed-to-gain ratio and about 10% was judged most suitable^[30]. That SMS contained 29.41% ash, illustrating why mineral dilution must be considered together with fibre. In vitro work on corn-based *P. ostreatus* SMS suggested that as much as 50% of corn silage could be replaced for beef and non-lactating dairy cattle^[37]. The contrast with the lower in vivo recommendation is instructive: batch culture gives no information about willingness to eat or long-term tolerance. Formulation complicates interpretation further. Adding urea and molasses to *P. ostreatus* SMS maintained nutrient utilisation up to 20% inclusion, whereas higher levels reduced digestibility and nitrogen retention^[38]. Gas-production tests can help screen materials before animal work^[39] but cannot detect refusal, sorting, or chronic effects. Future studies should separate the value of the residue itself from the value added by other ingredients.

Goat and deer studies reinforce the importance of diet design and dose. A fermented total mixed ration containing 30% *F. velutipes* residue improved daily gain, feed conversion, digestibility, selected immune measures, and feed cost in Guizhou black goats^[40], although only one ration was tested. In growing sika deer, replacing 10%–20% of concentrate with *P. ostreatus* SMS caused no major impairment, whereas 30% lowered haemoglobin and haematocrit^[41]. A later study combined SMS with a mixed microbial preparation and reported better growth and digestibility, but the contribution of SMS alone could not be determined^[42]. For ruminants, a moderate amount in a balanced ration is more defensible than substitution based on the low price of dry matter, and effective fibre, rumen-degradable nitrogen, ash, mineral balance, and storage stability all need to be considered.

The apparently conflicting ruminant results should not be interpreted as evidence that SMS has an intrinsically positive or negative effect on digestibility. Studies differ in mushroom species, original substrate, extent of fungal degradation, preservation method, additives, physical form, and the basis on which dietary inclusion is expressed. In vitro studies evaluate fermentability without capturing voluntary intake, sorting, mineral load, or long-term adaptation. For example, cellulase improved in vitro digestibility of *P. eryngii* SMS silage whereas lactic-acid bacteria mainly improved preservation^[35], and replacement of up to 50% of corn silage appeared feasible in vitro^[37], whereas approximately 10% was considered more suitable in an in vivo Hu-sheep study^[30]. Cultivation cycle and storage history may contribute further variation, but both factors are still poorly reported. Until they are described routinely, apparently conflicting findings are better interpreted in a source- and treatment-specific context rather than as evidence for or against a universal inclusion level.

4.2 Pigs

Pigs are more sensitive to high fibre and poor nutrient accessibility, and the clearest evidence supports low-dose functional use rather than bulk replacement of protein or energy. In weaned pigs, 0.5–1.5 g/kg *C. militaris* SMS improved final

body weight, average daily gain, feed intake, serum immunoglobulin A (IgA), and selected lipid and inflammatory measures at the highest dose, while reducing intestinal *Escherichia coli*^[43]. In growing pigs, 2 g/kg improved final weight, average daily gain, immunoglobulins, and antioxidant capacity, although feed intake and gain-to-feed ratio did not change significantly^[44]. These doses fall within the range normally used for functional supplements.

This distinction is mechanistically important. At low doses, accessible beta-glucans may influence intestinal morphology, immune signalling, and microbial communities, whereas at higher SMS inclusion the same fungal biomass also contributes insoluble chitin-glucan walls that dilute digestible nutrients and restrict access to intracellular protein. Evidence from purified or non-SMS beta-glucans should therefore be used to explain possible mechanisms rather than to assume that the intact, cross-linked chitin-glucan walls of SMS are equally bioaccessible^[45].

Higher inclusion has mainly been tested after microbial treatment. In weaned piglets, 3% *Bacillus*-fermented *L. edodes* residue changed hindgut microbial communities and increased tight-junction gene expression and serum immunoglobulins, but did not significantly improve growth^[46]. The product contained peptides, bacterial biomass, and microbial metabolites, so the active component is uncertain. Many fermentation studies report the inoculated organism and proximate composition but do not measure what happened to fungal beta-glucan, chitin, volatiles, or residual plant fibre. The wider literature on non-starch-polysaccharide enzymes in pigs shows a similar gap: in vitro digestibility may improve without a significant gain in nursery-pig performance^[47]. Enzymes alone are therefore unlikely to turn a fibre-rich SMS into a high-inclusion ingredient for young pigs. Trials should include faecal consistency, ileal digestibility, digesta viscosity, volatile fatty acids, biogenic amines, and intake across a dose series; a favourable microbiome change is not enough without evidence of nutrient use and tolerance.

4.3 Poultry

Poultry studies generally use much less SMS than ruminant studies. In 240 broilers, 0.50%–2% mushroom waste compost was tested, and the lowest dose improved feed conversion and influenced fat metabolism and antioxidant status^[48]. A recent review likewise describes current poultry use mainly as supplementation rather than replacement of energy or protein^[8]. Enzyme-microbe co-fermented *G. lucidum* SMS at 1.50% or 3% improved average daily gain and feed-to-gain ratio in yellow-feathered broilers, but apparent nutrient digestibility did not change significantly; 1.50% was recommended under the conditions tested^[49]. In a nutritionally poor broiler diet, 1.25%–5% *P. ostreatus* SMS only partly offset the effects of replacing soybean meal with marula seed cake and Mucuna seed meal^[50]. SMS cannot compensate for an imbalanced basal diet.

Particle size, insoluble fibre, mineral concentration, and the balance between immune-active polysaccharides and nutrient dilution are likely to shape the poultry response. The useful inclusion level may change when the right enzyme is added: broilers fed up to 8% wheat bran with xylanase maintained or improved daily gain and intake^[51]. The *G. lucidum* co-fermentation study is the closest test on an SMS containing both chitin and beta-glucan, and its growth benefit occurred without a measurable increase in apparent digestibility^[49]. Enzymatic or microbial treatment may therefore improve performance at low inclusion without proving that fungal and plant walls have been sufficiently opened. In layers, the

outcomes of interest also extend beyond growth. Dietary supplementation with 3% *G. lucidum* residue reduced ammonia and hydrogen sulfide emissions^[52], and a laying-duck study examined laying performance, egg quality, and serum biochemistry^[53]. These findings need confirmation with better source descriptions and dose-response designs.

4.4 Rabbits

Rabbits sit between ruminants and other monogastric animals: hindgut fermentation allows them to use fibrous ingredients, but palatability and energy density still matter. In growing rabbits, *P. ostreatus* SMS replaced 20%, 40%, or 60% of berseem hay. The 40%–60% treatments improved several digestibility coefficients, and the 60% treatment increased final body weight, raised caecal volatile fatty acids, and reduced feed cost^[54]. The denominator is important: SMS replaced part of the forage, not 60% of the complete diet. The study supports its use as a structured roughage in a balanced rabbit diet rather than unrestricted inclusion as a bulk ingredient. Caecal fermentation can therefore recover volatile fatty acids from structural carbohydrate that escapes small-intestinal digestion, which helps explain why rabbits can tolerate much greater replacement of the forage fraction than pigs or poultry, although energy density and palatability remain limiting.

4.5 Aquaculture, Insects, and Indirect Conversion

Fish studies also point mainly to low functional doses. Nile tilapia fed 5–40 g/kg *C. militaris* SMS showed the strongest combined response in growth, mucosal and serum immunity, and resistance to *Streptococcus agalactiae* (*S. agalactiae*) at 10 g/kg^[55]. Adding *Lactobacillus plantarum* (*L. plantarum*) to the same dose further improved feed conversion and immune measures^[56], although the two contributions could not be separated. *Agaricus*

blazei (*A. blazei*) SMS at 1%–5% also improved disease resistance in Nile tilapia^[57]. In contrast, a preliminary catfish study reported lower growth with SMS-based pellets than with a commercial feed, despite favourable survival and digestibility observations in very small groups^[58]. The evidence is stronger for functional supplementation than for replacement of protein or energy. High ash, poorly digested fibre, water stability, and strong flavour are especially important in extruded aquatic feeds, and no single inclusion range can be assumed across fish species. Fish should not be considered uniformly unable to digest chitin. In Nile tilapia and rainbow trout, dietary chitin from insect meal induced intestinal exochitinase activity and was partly digested, but apparent chitin and nutrient digestibility decreased as dietary chitin increased^[59]. Whether this extends to the cross-linked chitin-glucan walls of SMS remains untested, but the dose dependence supports the distinction between potentially useful structural carbohydrate at low exposure and an antinutritional burden at higher inclusion.

Materials that are hard to feed directly may instead be used to grow insects, microbial protein, or a second fungus. Black soldier fly larvae performed similarly to a vegetable control when SMS from three mushrooms made up 15% of the rearing substrate, whereas 30% reduced larval, prepupal, and pupal weights. Larval biomass nevertheless contained six-fold to ten-fold more protein than the raw SMS, and survival exceeded 99%^[60]. This result suggests that SMS can also dilute insect diets unless it is blended with a richer co-substrate. A recent model combines microbial depolymerisation, detoxification, and insect rearing to recover nitrogen and produce functional biomass^[5]. Such systems should be compared with direct enzyme treatment or ruminant feeding because every additional biological step loses some material, costs money, and introduces new safety questions. Selected feeding studies and their practical interpretation are summarised in **Table 2**.

Table 2. Selected animal studies and what they show for practical feed use

Animal / Stage	SMS Source and Original Substrate	Treatment and Dietary Inclusion	Main Finding	Interpretation
Hanwoo Steers	SMS; mushroom species and original substrate not specified	Microbial fermentation; supplementation level not reported	Feasible supplementation and economic potential ^[32]	Preservation improved; composition and intake still matter
Sheep	<i>A. bisporus</i> spent wheat-straw compost	Casing removed, no further treatment; 10%–30% of diet DM	Up to 20% maintained digestibility; 30% reduced intake and digestibility ^[29]	The workable level was below 30%
Holstein Finishing Calves	<i>A. bisporus</i> spent wheat-straw compost	Fed as mash or pellets; 15% of diet DM	Pelleted form supported better use than mash ^[31]	Physical form affected how well the material was used
Holstein Steers	<i>F. velutipes</i> SMS	Ensiled with whole-crop corn, with or without urea; fed as a silage-based diet	Lower rumen protozoa and enteric methane ^[36]	Applies to a formulated silage, not untreated SMS
Hu Sheep	<i>P. ostreatus</i> SMS (29.41% ash)	No treatment; replaced whole-plant corn silage at 5%–20% of diet DM	20% worsened feed-to-gain; approximately 10% was judged most suitable ^[30]	High ash may limit inclusion
Guizhou Black Goats	<i>F. velutipes</i> residue	Microbially fermented TMR; 30% of the ration	Improved growth, digestibility, feed conversion, and selected immune indices ^[40]	Only one inclusion level was tested
Growing Sika Deer	<i>P. ostreatus</i> SMS	No treatment; replaced 10%–30% of the concentrate	10%–20% caused no major impairment; 30% lowered haemoglobin and haematocrit ^[41]	Blood indices revealed a limit growth did not show
Young Sika Deer	SMS; mushroom species and original substrate not specified	Combined with a compound microbial preparation; inclusion level not reported	Improved growth, digestibility, and intestinal microbial indicators ^[42]	SMS and the microbial preparation cannot be separated

Table 2. Selected animal studies and what they show for practical feed use (continued)

Animal / Stage	SMS Source and Original Substrate	Treatment and Dietary Inclusion	Main Finding	Interpretation
Small Ruminants	<i>P. ostreatus</i> SMS	Supplemented with urea and molasses; graded inclusion up to and above 20% of diet DM	Up to 20% maintained nutrient utilisation; higher levels reduced digestibility and nitrogen retention ^[38]	Urea and molasses were added, so SMS alone is not responsible
Weaned Pigs	<i>C. militaris</i> SMS (grain-based)	No treatment; 0.50–1.50 g/kg of diet	The highest dose improved growth, IgA, and selected gut and serum indices ^[43]	Fits low-dose functional supplementation
Growing Pigs	<i>C. militaris</i> SMS (grain-based)	No treatment; 2 g/kg of diet	Improved final weight, ADG, immunoglobulins, and antioxidant capacity ^[44]	Consistent with a functional dose
Weaned Piglets	<i>L. edodes</i> cultivation residue	<i>Bacillus</i> fermentation; 3% of diet	Improved barrier and immune markers; growth differences were not significant ^[46]	Active component uncertain
Broilers	Mushroom waste compost; species not specified	No treatment; 0.50%–2% of diet	The lowest dose improved feed conversion and antioxidant/fat-metabolism indices ^[48]	More consistent with supplementation than bulk replacement
Yellow-Feathered Broilers	<i>G. lucidum</i> SMS	Enzyme-microbe co-fermentation; 1.50% or 3% of diet	Improved growth and feed-to-gain; digestibility was unchanged ^[49]	Mechanism of the growth response unclear
Broilers on a Challenging Diet	<i>P. ostreatus</i> SMS	No treatment; 1.25%–5% of a marula seed cake and Mucuna seed meal diet	Only limited mitigation of an imbalanced alternative-protein diet ^[50]	Cannot compensate for an unbalanced basal diet
Growing Rabbits	<i>P. ostreatus</i> SMS	No treatment; replaced 20%–60% of berseem hay, that is, of the forage fraction only	Higher replacement improved digestibility and caecal fermentation; 60% increased final weight ^[54]	Replaced the forage fraction, not 60% of the diet
Nile Tilapia	<i>C. militaris</i> SMS (grain-based)	No treatment; 5–40 g/kg of diet	10 g/kg produced the strongest combined growth, immune, and disease-resistance response ^[55]	Supports functional use, not bulk replacement
Nile Tilapia	<i>C. militaris</i> SMS (grain-based)	Combined with <i>L. plantarum</i> ; 10 g/kg of diet	Improved feed conversion and immune measures ^[56]	The two effects cannot be separated
Nile Tilapia	<i>A. blazei</i> SMS	No treatment; 1%–5% of diet	Improved immune responses and resistance to <i>S. agalactiae</i> ^[57]	Disease-resistance evidence is stronger than nutrient-replacement evidence
African Catfish	SMS; mushroom species and original substrate not specified	Formulated into pelleted feed; inclusion level not reported	Growth was lower than with a commercial feed, despite acceptable survival and preliminary digestibility data ^[58]	Negative result worth retaining; very small groups
Black Soldier Fly Larvae	SMS from three mushroom species	No treatment; 15% or 30% of the rearing substrate	15% maintained performance; 30% reduced developmental weights ^[60]	Insects also tolerated only a limited proportion of SMS

NOTE: SMS: spent mushroom substrate; DM: dry matter; TMR: total mixed ration; ADG: average daily gain; IgA: immunoglobulin A.

5. Targeted Bioprocessing: Beyond Generic Yeast or Lactic-Acid Fermentation.

Because the source determines the relative amounts and accessibility of plant and fungal wall material, processing should aim to modify the particular structural constraint that limits use in the intended animal.

5.1 Why Routine Yeast or Lactic-Acid Fermentation is Not Enough

Fermentation is often treated as a universal answer for agricultural by-products. The problem is that the word can refer to very different aims: preservation, acidification, microbial protein enrichment, plant-fibre breakdown, fungal-wall opening, detoxification, or flavour change. A routine inoculum cannot be expected to do all of them. *Saccharomyces* and many lactic-acid bacteria are useful for aroma formation, acidification, sugar consumption, and storage stability, but they do not provide a complete beta-1,3/1,6-glucanase-chitinase-mannanase system. Yeast also adds its own beta-glucan-, mannan-, and chitin-containing wall, which may account for about 15%–30% of cell dry mass^[61]. A

higher crude-protein percentage after yeast fermentation may therefore reflect added microbial biomass rather than release of protein trapped in the mushroom wall, and the total amount of insoluble wall polysaccharide may even increase.

The microorganism must be chosen at the strain level. Genomic analysis of *Kluyveromyces marxianus* SHY2 identified many genes associated with proteolysis, but the authors cautioned that annotation can overestimate actual proteolytic activity and must be checked experimentally^[62]. The same applies to SMS fermentation. A genus or species name does not show that a strain produces enough beta-glucanase, chitinase, protease, or lignocellulase, changes flavour in a useful way, or is safe under the intended process conditions.

5.2 Start by Identifying What Limits the Material

Routine batch checks can include source traceability, dry matter or water activity, crude protein, neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), ash, key minerals, pH, microbial counts, mycotoxins, and obvious abnormalities in smell or appearance. Product development may require additional measurements, including amino-acid composition, beta-glucan,

chitin or glucosamine, fungal-biomass markers, reducing sugars, organic acids, in vitro digestion, viscosity or water-holding behaviour, and selected volatiles. Routine release tests should remain practical, while more demanding analyses can be used to establish specifications for a particular source. The original substrate also matters: cottonseed-hull materials, manure-based composts, and chemically treated substrates require different safety checks.

Fermentation cannot correct excessive ash; separation, washing, blending, or exclusion from feed may be more appropriate. A material rich in fungal biomass may require fungal-cell-wall-degrading enzymes, such as beta-glucanases and chitinases, whereas one dominated by residual lignocellulose may require cellulase and xylanase. Strong mushroom odour or bitterness calls for strains selected for flavour change and should be checked in an intake test. Wet, unstable SMS must be ensiled, dried, or acidified promptly.

The choice of microorganism or enzyme should therefore follow the dominant limitation of the SMS matrix. Fungal-biomass-rich materials require strains or enzyme preparations with demonstrated beta-glucanase, chitinase, or related wall-remodelling activities, whereas lignocellulose-dominated materials require cellulolytic and hemicellulolytic activity matched to the residual plant substrate. Proteolysis may be useful after wall accessibility has been improved. Strains intended for flavour improvement should be selected according to measured changes in relevant volatiles or bitter compounds, whereas lactic-acid bacteria used primarily for preservation should be judged by acidification, aerobic stability, and suppression of spoilage rather than assumed wall-degrading capacity.

5.3 Opening the Fungal Cell Wall

The wall structure determines which enzymes are needed. Endo-beta-1,3-glucanase and beta-1,6-glucanase act on the glucan scaffold and cross-links; chitinase and N-acetylglucosaminidase act on chitin; and mannanase or endo-N-acetyl-beta-D-glucosaminidase (ENGase) may improve access through mannoprotein-rich outer layers. Proteases are likely to work better after the wall has become more permeable.

Several recent studies indicate why this approach is plausible. Increasing the expression of an endogenous wall-remodelling enzyme in *C. militaris* produced biomass with fewer residual solids, lower viscosity, and greater amino-acid release during simulated digestion^[25]. Treating *P. eryngii* with cell-wall-degrading enzymes increased protein extraction and shifted digestion products towards shorter peptides and free amino acids^[26], and a related study reported greater intestinal peptide release^[63]. All of these experiments used mycelium or fruiting bodies rather than SMS. In spent substrate, fungal biomass is dispersed through wet lignocellulosic material, which may reduce enzyme access and compete for enzyme activity. Fungal-wall treatment is therefore well supported in principle but still needs to be tested directly on clearly described SMS. ENGase remains a promising means of improving access through the outer wall, but it is not yet a routine feed-processing enzyme.

Complete hydrolysis of the wall is rarely desirable. Excessive treatment may release too much soluble sugar, increase osmotic load, or encourage unwanted fermentation during storage. The wall only needs to be opened enough to improve nutrient release while retaining an appropriate amount of structural fibre and potentially useful oligosaccharides for the intended animal.

5.4 Opening Residual Plant Cell Walls

Plant-wall treatment must reflect the original substrate. Cellulase alone may be insufficient when xylan, lignin, acetyl

groups, or silica restrict access. Cellobiohydrolase, endoglucanase, beta-glucosidase, xylanase, arabinofuranosidase, and esterases should be combined according to the material that remains after mushroom cultivation.

Work on *H. marmoreus* SMS showed that efficient hydrolysis required a balanced cellulase-xylanase mixture rather than a single enzyme. The optimised combination hydrolysed about 55% of the SMS, and engineered *C. militaris* then consumed about 50%–60% of its cellulose and hemicellulose^[3]. Although the product was pentostatin rather than feed, the study shows that missing enzyme activities and their proportions can matter more than total enzyme dose. Similar results have been reported for *P. eryngii* SMS. An optimised mixture of cellulase, xylanase, and beta-galactosidase maximised reducing-sugar release, and combining it with *Bacillus subtilis*, *Pediococcus acidilactici*, and *Saccharomyces cerevisiae* degraded 23.69% of neutral detergent fibre and increased nutrient content more than either treatment alone^[64]. A defined microbial consortium may be cheaper than purified enzymes and can provide complementary activities; constructed cellulose-degrading consortia have degraded SMS through the combined action of their members^[65]. The trade-off is control: a consortium may reduce enzyme cost but is harder to keep consistent among batches.

Physical or chemical pretreatment can reduce the amount of enzyme needed, but it must remain safe and affordable for feed. Grinding, extrusion, steam, ultrasound, high-pressure homogenisation, or mild alkali may improve enzyme access. Washing can remove soluble salts but also loses nutrients and creates wastewater. Fibre disappearance alone is a poor criterion, because degradation of structural carbohydrate may also be accompanied by dry-matter loss; treatment should therefore be evaluated by the recovery of degradable organic matter and, ultimately, by improved nutrient availability in the intended animal^[18].

5.5 Downstream Processing after Cell-Wall Opening

After wall opening, limited proteolysis may increase soluble protein and small peptides. However, intermediate hydrolysis can increase bitterness, whereas excessive release of free amino acids may favour biogenic-amine formation during uncontrolled fermentation or storage^[66,67]. Proteolysis should therefore be evaluated by peptide-size distribution, free amino nitrogen, bitterness, biogenic amines, nitrogen recovery, and animal digestibility.

Flavour treatment matters only if animals accept more of the resulting feed. Headspace solid-phase microextraction with gas chromatography-mass spectrometry (GC-MS) can measure the C8 compounds, oxidation products, acids, esters, and musty notes described in Section 3.4. The same batch should be analysed before and after treatment, and an electronic nose can help screen larger numbers of samples. The critical test is still a short preference or intake study in the intended animal. Selected microorganisms may transform undesirable volatiles by oxidation, reduction, or esterification, but this has not been tested systematically for the C8 alcohols and ketones that dominate mushroom aroma. Fermentation can also create excessive acidic, alcoholic, phenolic, or musty notes. Candidate strains need to remove unwanted odours without creating new ones. Aeration also needs to be controlled because many volatile transformations depend on redox conditions.

Lactic-acid bacteria remain useful for preservation, acidification, and suppression of spoilage organisms. They should not be described as a means of deeply opening the fungal wall unless the necessary enzyme activities have been measured. Yeast may help with selected aroma changes or oxygen removal, but the same caution applies.

No single process sequence will suit every SMS. Some materials require only preservation, whereas others may need washing, targeted enzymes, limited proteolysis, or selected fermentation. Enzymatic and microbial treatments can be complementary^[68,69], but combining all functions in one stage may impose incompatible pH, temperature, and

oxygen conditions. The simplest treatment that resolves the dominant limitation is therefore preferable; material that remains unsuitable for feed may instead be used for insects or microbial protein^[5]. **Table 3** links the main limitations of SMS to relevant measurements and possible treatments.

Table 3. Choosing treatments for the main limitations of SMS

Main Limitation	What to Measure	Possible Treatment	What Would Count as Improvement	Main Trade-Off or Limitation
Protein Trapped within Fungal Walls	Chitin/glucosamine, beta-glucan, soluble protein, residual solids	Beta-glucanase and chitinase; ENGase as an emerging option; a verified wall-lytic strain; physical assistance where needed	More accessible and digestible protein without excessive release of soluble sugar	Enzyme cost is high; over-treatment releases excess soluble sugar and may destroy potentially functional glucans
Residual Plant Cell Walls	NDF, ADF, ADL, cellulose/xylan profile, in vitro degradability	A cellulase-xylanase mixture matched to the substrate; extrusion, steam, or mild alkali where justified	Greater digestibility and energy value in the intended animal	Fibre disappearance does not always raise digestible energy; harsh pretreatment adds cost and safety concerns
Excess Ash, Salts, or Minerals	Ash, calcium, sodium, conductivity, substrate formulation	Separation, washing, blending, or no feed use	Mineral concentrations within limits for the intended animal	Washing loses nutrients and generates wastewater; blending dilutes minerals but does not remove them
Strong Mushroom, Musty, or Woody Odour	GC-MS volatiles, electronic nose, preference testing	A strain selected for useful flavour change; controlled aeration; adsorption or blending where justified	Lower unwanted volatiles together with better feed acceptance	Fermentation may create new acidic or musty notes; volatile data alone do not predict intake
Bitterness or an Undesirable Peptide Profile	Electronic tongue, bitter amino acids or peptides, sensory screening	Limited proteolysis, peptide trimming, or selected flavour fermentation	Better preference without loss of digestible protein	Intermediate hydrolysis can increase bitterness, and excess free amino acids favour biogenic-amine formation
High Spoilage Risk	Moisture, water activity, microbial counts, temperature history	Rapid ensiling or acidification, drying, hygienic pelleting	Stable storage, low pathogen and mycotoxin risk, and a defined shelf life	Preservation does not remove mycotoxins already present; drying adds energy cost
Large Differences Among Batches	Variation in NIR and chemical composition	Separate batches, blend when appropriate, use rapid spectroscopy, and set practical limits	Nutrient composition and safety that remain predictable from batch to batch	Rapid spectroscopy needs source-specific calibration; blending evens out variation but obscures batch identity

NOTE: SMS: spent mushroom substrate; NDF: neutral detergent fibre; ADF: acid detergent fibre; ADL: acid detergent lignin; GC-MS: gas chromatography-mass spectrometry; ENGase: endo-N-acetyl-beta-D-glucosaminidase; NIR: near-infrared spectroscopy.

6. Safety, Quality Control, and Standardisation

Using SMS as feed places it in the food chain and requires stricter control than use in compost, materials, or energy. An edible cultivation substrate does not guarantee that the spent material is feed-safe. Risks may come from the original ingredients, supplements, disinfectants, casing soil, competing moulds, storage, or uncontrolled secondary fermentation.

These concerns should not be treated as equivalent. Mycotoxins, pathogenic microorganisms, pesticide or disinfectant residues, and excessive concentrations of regulated contaminants are safety hazards that may require rejection of a batch. High ash, salinity, or calcium are more often formulation constraints because their effects depend on the amount of SMS included and the mineral balance of the complete diet. Cottonseed-derived substrates require source-specific attention to residual free gossypol, but carryover should be measured rather than assumed because fungal cultivation can also degrade gossypol. Strong mushroom, musty, woody, or bitter notes are primarily palatability concerns unless a specific hazardous compound is identified. Distinguishing these categories is important because they require different control measures.

A feed specification should include traceability to the mushroom farm and substrate recipe; confirmation that the material came from a completed, normal fruiting cycle; rejection

of batches with visible spoilage, abnormal heating, lost traceability, or suspected chemical contamination; moisture and water activity; total aerobic and fungal counts; *Salmonella* and other relevant pathogens; mycotoxins; pesticide and disinfectant residues; ash and mineral limits; and, where appropriate, cottonseed-associated factors or other substrate-specific risks. Heavy metals deserve proportionate attention rather than alarm. Agricultural biomass can bring cadmium, lead, and mercury into mushroom cultivation, and fruiting bodies can accumulate them^[70]. In traceable factory production, where straw, sawdust, and other major ingredients are checked before use, the risk in SMS should usually be low enough for periodic verification. More frequent testing is warranted when the source is uncertain, substrates come from contaminated areas, or production is small and poorly documented. Heavy metals should not be presented as a universal obstacle to feed use. Antibiotic-resistance genes and microbial ecology also deserve attention when fermented products are intended to contain live microorganisms.

High ash and mineral imbalance are practical concerns. The Hu-sheep study used *P. ostreatus* SMS with nearly 30% ash and observed poorer feed efficiency at the highest inclusion^[30]. The result cannot be assigned solely to ash, but it shows why source composition and inclusion level must be reported together. Similar caution applies to calcium-rich *Agaricus* compost and SMS containing lime, gypsum, or casing residues.

Control measures should likewise be matched to the risk. Washing can reduce soluble salts and some water-soluble compounds, but it also removes nutrients and generates wastewater. Blending can reduce the concentration of ash or minerals in the final diet but does not remove a hazardous contaminant. Drying, ensiling, or acidification can improve storage stability and suppress further spoilage, but they should not be assumed to eliminate mycotoxins already present. Extrusion or other physical treatments may improve handling, hygiene, and enzyme accessibility, but they are not universal detoxification steps. Enzyme treatment is appropriate when poor nutrient accessibility is caused by plant or fungal cell walls, whereas selected fermentation should only be considered a detoxification treatment when the relevant compound has been measured before and after processing and the responsible activity has been demonstrated. Material that remains chemically or microbiologically unsafe should be excluded from feed rather than processed in an attempt to rescue it.

Quality control should not stop at a single certificate of analysis. Near-infrared spectroscopy, image analysis, and rapid chemical methods can help classify batches, but the models must be calibrated with reference data from the relevant SMS source. Reports should include mushroom species and, where available, strain; the original substrate percentages; harvest cycle; treatment; dry-matter loss; complete proximate and fibre analyses; fungal-wall markers; safety results; and the exact basis on which dietary inclusion was calculated.

Two checks are especially important and are often missing. The first is a dry-matter and nutrient balance. Percentage composition can be misleading when fermentation removes carbon as carbon dioxide or washing transfers soluble nutrients to wastewater. Studies should report initial and final dry mass, recovery of protein and amino acids, changes in structural carbohydrate, water and energy inputs, and the amount and composition of side streams. These data show whether nutrients were genuinely released or merely

concentrated by dry-matter loss. The second is storage testing of the finished product. Fermentation pH alone does not establish shelf life. Re-heating, aerobic stability after opening, pathogen regrowth, mycotoxin formation, volatile changes, pellet durability, and nutrient losses should be measured under realistic farm conditions. A product is not standardised if it changes unpredictably between factory release and feeding.

7. From Source Information to Practical Feed Use

The following four grades are offered as a conceptual framework for organising the evidence discussed above, not as a validated classification. They summarise how much treatment an SMS may need and should not be read as permanent labels. Material that is clearly unsafe or cannot be traced should be excluded before grading. Among the remaining materials, Grade A is stable, low-risk, nutritionally adequate, and acceptable enough for direct low-level inclusion in the intended animal. Grade B is suitable after straightforward preparation such as preservation, grinding, blending, or ensiling. Grade C still contains useful nutrients but needs a specific treatment, for example fungal-wall or plant-wall enzymes, washing to reduce salts, or fermentation selected to change flavour. Grade D is reserved for traceable material that remains technically or economically unsuitable for feed after assessment. No numerical thresholds are proposed here, because the available studies do not yet provide the batch-level compositional data or the dose-response feeding trials needed to define them. The boundaries will also differ among animals. The framework should therefore be treated as a hypothesis to be tested: its usefulness depends on whether measured batch characteristics predict animal performance, and it will need revision as such data accumulate. **Figure 3** summarises how source information, treatment choice, intended use, and animal testing fit together.

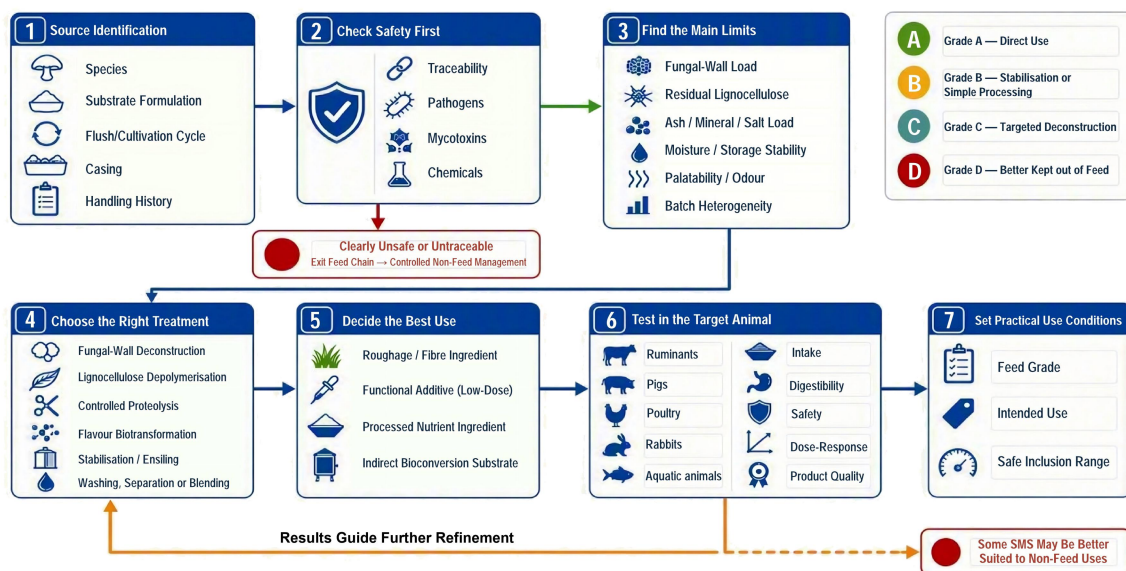


Figure 3 Practical development of SMS biofeed. Source information and safety screening come first. The main limitations of each batch then guide the choice of treatment and the most realistic use. Animal studies are used to assess intake, digestibility, safety, dose-response, and product quality and to define a suitable inclusion range. Results may lead to changes in treatment or intended use, and some materials will be better suited to non-feed applications. Grades A-D represent a research hypothesis for the amount of preparation required; they are not validated feed standards and should be tested against source-resolved composition, intake, digestibility, safety, and dose-response data for the intended animal species. **NOTE:** SMS: spent mushroom substrate. Copyright: © 2026 The Authors. Figure assembled and edited by the authors using Microsoft PowerPoint (version 16.0); some visual elements were generated with Microsoft Copilot and further refined by the authors. The figure is not reproduced or adapted from any previously published source.

Feed grade and intended use are related but are not the same question. A material may serve as a roughage or fibre ingredient for ruminants and rabbits, a low-dose functional additive, a processed nutrient ingredient after wall opening and protein release, or a substrate for insects, microbial protein, or a second fungal cultivation. Both the grade and the intended use are animal-specific: a fibrous material may need only simple preparation for adult ruminants but extensive treatment, or no feed use at all, for weaned pigs or fish, and an SMS rich in beta-glucan and mycelium may be valuable at a low dose yet poor as a bulk energy ingredient. Feed value belongs to the combination of material, treatment, dose, diet, and animal; it is not an intrinsic property of SMS alone.

Economic assessment should use measures such as cost per kilogram of digestible protein, metabolisable energy, or animal product rather than the purchase price of wet SMS. Drying, transport, enzymes, wastewater treatment, quality testing, and fermentation losses can erase the apparent advantage of a residue that is free at the farm gate. Co-location in mushroom industrial parks can reduce transport and allow shared use of heat, water, enzymes, and residual biomass^[1], but treatment still has to remain inexpensive.

Prospective validation should first exclude untraceable or unsafe batches and then test whether source-resolved indicators — including storage stability, ash and mineral load, plant- and fungal-wall fractions, protein accessibility and digestibility, palatability and voluntary intake, and animal dose-response — predict the amount of processing required. Grade boundaries should then be derived from whether a safe batch meets predefined nutritional and animal-performance criteria directly (Grade A), after simple preparation (Grade B), only after targeted treatment (Grade C), or remains unsuitable after technically and economically reasonable treatment (Grade D).

8. Future Perspective

A source-resolved database is needed that links mushroom species, original substrate, cultivation cycle, composition, treatment, dose, and animal response. Existing studies are difficult to compare because SMS is used as a single name for many materials. Conventional feed tables offer a useful model: the INRAE-CIRAD-AFZ database reports the number of observations, standard deviation, minimum, and maximum for each ingredient, allowing formulators to see variability rather than only a mean value^[71]. An SMS database should likewise require mushroom species, substrate formulation, flush number, and post-harvest history. The studies that populate it would benefit from clearer reporting. ARRIVE 2.0 identifies the design, sample size, and statistical details most often omitted from animal studies, and wider use would make SMS trials easier to compare^[72].

Processing conditions need the same discipline. Enzyme loading, solids concentration, pH, temperature, treatment time, and moisture or solid-to-liquid ratio can strongly affect the outcome, as can inoculum size, fermentation time, aeration, and final pH for microbial treatments. These parameters should be reported together with dry-matter recovery and the basis used to express enzyme dosage, because conditions cannot otherwise be transferred reliably among SMS sources.

The dual cell-wall concept also needs direct testing. Studies should measure plant-wall and fungal-wall fractions before and after

treatment and relate the changes to protein accessibility, viscosity, energy value, and hindgut fermentation. Solid-state nuclear magnetic resonance (NMR) can provide structural information on cell-wall polysaccharides, but overlapping carbohydrate resonances limit direct separation of fungal- and plant-derived signals in mixed SMS. It should therefore be combined with fungal-biomass markers such as chitin/glucosamine or ergosterol and complementary plant-wall analyses^[22,73,74]. Ergosterol itself requires source-specific calibration because it reflects membrane sterol rather than wall polymer. Animal measurements can then show whether chemical changes matter. In broilers, moderate differences in soluble non-starch polysaccharide altered ileal viscosity, caecal short-chain fatty acids, and intestinal microbiota^[75], providing a relevant set of physiological responses for testing whether changes in plant- and fungal-wall accessibility translate into measurable effects in SMS-fed animals.

Measured activity, rather than the name of the microorganism, should guide culture selection. Useful screening would include beta-1,3/1,6-glucanase, chitinase, ENGase, xylanase, cellulase, protease, flavour-changing capacity, absence of toxin production, and performance at realistic solids concentrations. When a natural isolate lacks a required activity, synthetic-biology tools may allow edible fungi or their enzyme systems to be improved for wall opening or flavour change^[76]. The caution from the *K. marxianus* study still applies: a predicted gene is a reason to test a strain, not evidence that it will work in an industrial fermentation^[62].

Palatability deserves its own experiments. Double-choice feeding, in which animals choose between a test and reference diet, has been used to rank ingredients in pigs and can detect preferences within the first few days^[77]. Preference and voluntary intake are not identical, so short-term choice tests should be read together with longer intake and performance measurements^[78]. For SMS, preference tests should be combined with electronic-nose profiles and targeted volatile analysis to determine whether a treatment reduces mushroom, bitter, or musty notes and whether animals eat more.

Animal trials also need dose-response designs and full reporting of negative or null findings. Experimental unit, replication, and power calculations should be defined before the study begins^[79]. Publication bias towards significant outcomes is well documented^[80], yet doses that provide no benefit or cause harm are essential for feed formulation. Trials should report intake, feed efficiency, faecal consistency, nutrient digestibility, blood and organ safety, product quality, and adaptation over time.

Processing also needs to preserve useful functions. Beta-glucans, chitin-derived oligosaccharides, organic acids, and fungal metabolites may support animal health, but their effects depend on dose and structure. Molecular weight, branching, solubility, and chain conformation influence the immunomodulatory activity of glucans, so materials from different sources cannot be assumed equivalent^[81]. Excessive hydrolysis may destroy structures that contribute to a functional response, while insufficient treatment leaves nutrients inaccessible. The retained and released fractions should be measured rather than assumed.

Economic and environmental comparisons should include direct ruminant feeding, enzyme treatment, selected fermentation, insect conversion, and non-feed uses. The best option will depend on the SMS source, local markets, transport distance, and available utilities, and a combination of uses will often recover more value

than a single solution.

9. Conclusion

Spent mushroom substrate is abundant, but it is not a ready-made feed ingredient. Its value depends on the original cultivation substrate, the fungal biomass formed during production, storage history, and the animal that will receive it. Residual plant walls limit energy release, fungal walls restrict access to intracellular protein, and flavour, bitterness, minerals, moisture, and spoilage can further reduce acceptance or safety. Feeding studies support moderate roughage use in ruminants and rabbits and low-dose functional use in pigs, poultry, and fish, but no single inclusion level or treatment fits every SMS. The proposed four-grade scheme should therefore be regarded as a research hypothesis to be validated prospectively against source-resolved composition, safety, intake, digestibility, and dose-response data rather than as an established feed standard. A practical approach is to know the source, exclude unsafe material, identify the main limitations, choose the simplest treatment that addresses them, and test the result in the intended animal. Used in this way, SMS could become an important high-volume feed resource without forcing every residue into the same application.

Author Contributions

Conceptualization, Hongyu Chen and Gen Zou; methodology, Ying Wang; investigation, Ying Wang and Hongyu Chen; data curation, Ying Wang, Hongyu Chen and Youran Shao; resources, Bing Li; validation, Bing Li; writing — original draft preparation, Hongyu Chen; writing—review and editing, Ying Wang, Bing Li, Youran Shao and Gen Zou; visualization, Hongyu Chen and Youran Shao; supervision, Gen Zou; project administration, Gen Zou; funding acquisition, Gen Zou. All authors have read and agreed to the published version of the manuscript.

Artificial Intelligence (AI) Statement

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with language editing and figure-text refinement and Microsoft Copilot to generate selected visual elements used in the figures. All scientific content, interpretations, references, figure composition, and final wording were reviewed and approved by the authors, who take full responsibility for the manuscript.

Data Availability

No new datasets were generated for this review.

Ethics Approval

Not applicable. This review did not involve human participants or animal experimentation.

Conflicts of Interest

The authors declare no conflict of interest.

Funding

This work was supported by the National Key Research and

Development Program of China (2023YFF1000800) and the Excellent Team Plan of Shanghai Academy of Agricultural Sciences (2022)014.

References

- [1] Wang Y, Chen W, Li F, *et al.* Spent mushroom substrate substitutes silkworm pupae powder in the industrial cultivation of *Cordyceps militaris*: Agronomic validation, mechanism, and cost implications. *Industrial Crops and Products*. 2026, 248: 1-12. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [2] Martin C, Xiong S, Zervakis GI. Exploiting the biorefinery potential of spent mushroom substrate: The time to do it is now. *Molecules*. 2025, 30 (23): 1-10. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [3] Zou G, Li B, Wang Y, *et al.* Efficient conversion of spent mushroom substrate into a high value-added anticancer drug pentostatin with engineered *Cordyceps militaris*. *Green Chemistry*. 2021, 23 (24): 10030-10038. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [4] Ma X, Yan S, Wang M. Spent mushroom substrate: A review on present and future of green applications. *Journal of Environmental Management*. 2025, 373: 1-15. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [5] Yuan R, Ma X, Ma X, Jia X, Shen S. Circular biomanufacturing of non-grain feeds: Synergizing microbial fermentation and insect bioreactors for animal health. *Biofeed Science and Technology*. 2026, 1 (1): 1-12. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [6] Zhang Y, Zheng A, Chen Z, *et al.* Research Progress on Feed Resources of *Zanthoxylum bungeanum* By-Products. *Biofeed Science and Technology*. 2026, 1 (1): 1-7. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [7] Leong YK, Ma TW, Chang JS, *et al.* Recent advances and future directions on the valorization of spent mushroom substrate (SMS): A review. *Bioresource Technology*. 2022, 344: 1-13. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [8] Baptista F, Almeida M, Paie-Ribeiro J, *et al.* Unlocking the Potential of Spent Mushroom Substrate for Enhanced Agricultural Sustainability: From Environmental Benefits to Poultry Nutrition. *Life*. 2023, 13: 1-18. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [9] Martin C, Zervakis GI, Xiong S, *et al.* Spent substrate from mushroom cultivation: exploitation potential toward various applications and value-added products. *Bioengineered*. 2023, 14 (1): 1-40. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [10] Mortada AN, Bolhassan MH, Wahi R. Physicochemical composition of spent oyster mushroom substrate. *Malaysian Journal of Analytical Sciences*. 2020, 24 (6): 848-854. [\[Google Scholar\]](#)
- [11] Akter A, Klausen SJ, Romero-Soto LA, *et al.* Comparative extraction of bioactive compounds from spent mushroom substrates of *Lentinula edodes* and *Pleurotus ostreatus* using subcritical water and pressurized ethanol. *Industrial Crops and Products*. 2025, 235: 1-17. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [12] Yague MR, Gonzalez-Perez JA, Almendros G, *et al.* Assessing the Potential Agronomic Value of Spent Mushroom Substrates: Evaluating Their Suitability to Contribute to Soil Carbon Storage. *Sustainability*. 2025, 17 (16): 1-19. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [13] Guo Y, Zhang Y, Zhang D, *et al.* Bioactive metabolites in *Hypsizygus marmoreus* spent substrate enhanced *Pleurotus ostreatus* growth. *Bioresource Technology*. 2025, 442: 1-15. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [14] van Kuijk SJA, Sonnenberg ASM, Baars JJP, *et al.* Fungal treatment of lignocellulosic biomass: Importance of fungal species, colonization and time on chemical composition and *in vitro* rumen degradability. *Animal Feed Science and Technology*. 2015, 209: 40-50. [\[Google Scholar\]](#) [\[CrossRef\]](#)

Hongyu Chen *et al.* *Biofeed Science and Technology*. 2026, 01 (2): 1-17.

- [15] Nayan N, Sonnenberg ASM, Hendriks WH, *et al.* Screening of white-rot fungi for bioprocessing of wheat straw into ruminant feed. *Journal of Applied Microbiology*. 2018, 125 (2): 468-479. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [16] Nayan N, van Erven G, Kabel MA, *et al.* Improving ruminal digestibility of various wheat straw types by white-rot fungi. *Journal of the Science of Food and Agriculture*. 2019, 99 (2): 957-965. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [17] Martens SD, Wildner V, Zeyner A, *et al.* In vitro ruminal degradability of wheat straw cultivated with white-rot fungi adapted to mushroom farming conditions. *Scientific Reports*. 2023, 13: 1-9. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [18] Sonnenberg ASM, Nayan N, Cone JW, *et al.* Intraspecies Variation Offers Potential to Improve White Rot Fungi for Increasing Degradability of Lignocellulose for Ruminants. *Journal of Fungi*. 2024, 10 (12): 1-11. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [19] Zhang L, Cheah IKM, Lu Y, *et al.* Characterization of Bioactive Compounds in Spent mushroom Substrate: A Metabolomic Perspective on Its Untapped Potential. *Foods*. 2026, 15 (1): 1-16. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [20] Daniso E, Comuzzo P, Spanghero M. Enzymatic activity of variously preserved extracts from spent mushroom substrate (*Pleurotus ostreatus*) and in vitro rumen degradation of extract-treated wheat straw. *Bioresources and Bioprocessing*. 2026, 13: 1-10. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [21] Gow NAR, Latge JP, Munro CA. The Fungal Cell Wall: Structure, Biosynthesis, and Function. *Microbiology Spectrum*. 2017, 5 (3): 1-25 [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [22] Kang X, Kirui A, Muszynski A, *et al.* Molecular architecture of fungal cell walls revealed by solid-state NMR. *Nature Communications*. 2018, 9: 1-12. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [23] Gow NAR, Lenardon MD. Architecture of the dynamic fungal cell wall. *Nature Reviews Microbiology*. 2023, 21: 248-259. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [24] Colosimo R, Warren FJ, Finnigan TJA, *et al.* Protein bioaccessibility from mycoprotein hyphal structure: In vitro investigation of underlying mechanisms. *Food Chemistry*. 2020, 330: 1-10. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [25] Wang Y, Chen W, Chen H, *et al.* Editing a mushroom with high-digestibility using a novel *endo-N-acetyl-β-D-glucosaminidase*. *International Journal of Biological Macromolecules*. 2025, 305: 1-11. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [26] Zhang M, Wang D, Liu H, *et al.* Unlocking sustainable protein from *Pleurotus eryngii*: Enzymatic degradation of cell wall enhances digestibility in mycelia and fruiting bodies. *LWT - Food Science and Technology*. 2025, 237: 1-13. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [27] Chakraborty N, Banerjee A, Sarkar A, *et al.* Mushroom Polysaccharides: A Potent Immune-Modulator. *Biointerface Research in Applied Chemistry*. 2021, 11 (2): 8915-8930. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [28] Wnuk S, Kinastowski S, Kaminski E. Synthesis and analysis of 1-octen-3-ol, the main flavour component of mushrooms. *Food/Nahrung*. 1983, 27 (5): 479-486. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [29] Fazaali H, Talebian Masoodi AR. Spent Wheat Straw Compost of *Agaricus Bisporus* Mushroom as Ruminant Feed. *Asian-Australasian Journal of Animal Sciences*. 2006, 19 (6): 845-851. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [30] Lu ML, Yuan GH, Rehemujiang H, *et al.* Effects of spent substrate of oyster mushroom (*Pleurotus ostreatus*) on ruminal fermentation, microbial community and growth performance in Hu sheep. *Frontiers in Microbiology*. 2024, 15: 1-16. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [31] Fazaali H, Shafyee-Varzeneh H, Farahpoor A, Moayyer A. Recycling of mushroom compost wheat straw in the diet of feedlot calves with two physical forms. *International Journal of Recycling of Organic Waste in Agriculture*. 2014, 3: 1-8. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [32] Kim YI, Lee YH, Kim KH, *et al.* Kwak WS. Effects of supplementing microbially-fermented spent mushroom substrates on growth performance and carcass characteristics of Hanwoo steers: A field study. *Asian-Australasian Journal of Animal Sciences*. 2012, 25 (11): 1575-1581. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [33] Baek YC, Jeong JY, Oh YK, *et al.* Effects of applying microbial additive inoculants to spent mushroom substrate (*Flammulina velutipes*) on rumen fermentation and total-tract nutrient digestibility in Hanwoo steers. *Korean Journal of Organic Agriculture*. 2017, 25 (3): 569-586. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [34] Kwak WS, Jung SH, Kim YI. Broiler litter supplementation improves storage and feed-nutritional value of sawdust-based spent mushroom substrate. *Bioresource Technology*. 2008, 99 (8): 2947-2955. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [35] Yi Q, Wang P, Tang H, *et al.* Fermentation Quality, In Vitro Digestibility, and Aerobic Stability of Ensiling Spent Mushroom Substrate with Microbial Additives. *Animals*. 2023, 13 (5): 1-18. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [36] Rangubhet KT, Mangwe MC, Mlambo V, *et al.* Enteric methane emissions and protozoa populations in Holstein steers fed spent mushroom (*Flammulina velutipes*) substrate silage-based diets. *Animal Feed Science and Technology*. 2017, 234 : 78-87. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [37] Anotaenwere CC, Isikhuemhen OS, Dele PA, *et al.* Ensiled *Pleurotus ostreatus* based spent mushroom substrate from corn: *In vitro* gas production, greenhouse gas emissions, nutrient degradation, and ruminal fermentation characteristics. *AIMS Microbiology*. 2025, 11 (1): 1-21. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [38] Crisostomo JCA, Manlapig JJD, Pascua MP, *et al.* In Vivo and In Vitro Digestibility and Fermentation Characteristics of Spent Mushroom Substrate (*Pleurotus ostreatus*) as a Feed Resource for Small Ruminants. *Animal Science Journal*. 2025, 96 (1): 1-13. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [39] Palangi V, Kaya A, Kaya A, *et al.* Ecofriendly Usability of Mushroom Cultivation Ssubstrate as a Ruminant Feed: Anaerobic Digestion Using Gas Production Techniques. *Animals*. 2022, 12 (12): 1-9. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [40] Long Y, Xiao W, Zhao Y, *et al.* Effects of *Flammulina velutipes* mushroom residues on growth performance, apparent digestibility, serum biochemical indicators, rumen fermentation and microbial of Guizhou black goat. *Frontiers in Microbiology*. 2024, 15: 1-14. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [41] Yuan C, Wu M, Chen X, *et al.* Growth Performance and Hematological Changes in Growing Sika Deers Fed With Spent Mushroom Substrate of *Pleurotus Ostreatus*. *Animals*. 2022, 12 (6): 1-10. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [42] Shi H, Hou T, Li Y, *et al.* Effects of Spent Mushroom Substrates and Compound Microorganism Preparation on the Growth Performance, Hematological Changes, and Intestinal Microbiota of Young Sika Deer.

- Animals*. 2025, 15 (16): 1-17. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [43] Boontiam W, Wachirapakorn C, Phaengphairee P, *et al.* Effect of Spent Mushroom (*Cordyceps militaris*) on Growth Performance, Immunity, and Intestinal Microflora in Weaning Pigs. *Animals*. 2020, 10 (12): 1-12. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [44] Boontiam W, Wachirapakorn C, Wattanachai S. Growth performance and hematological changes in growing pigs treated with *Cordyceps militaris* spent mushroom substrate. *Veterinary World*. 2020, 13 (4): 768-773. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [45] Luo J, Liu S, Yu B, *et al.* Beta-glucan from *Agrobacterium* sp. ZX09 improves growth performance and intestinal function in weaned piglets. *Journal of Animal Physiology and Animal Nutrition*. 2019, 103 (6): 1818-1827. [\[Google Scholar\]](#) [\[CrossRef\]](#).
- [46] Qi Q, Peng Q, Tang M, *et al.* Microbiome Analysis Investigating the Impacts of Fermented Spent Mushroom Substrates on the Composition of Microbiota in Weaned Piglets Hindgut. *Frontiers in Veterinary Science*. 2020, 7: 1-11. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [47] Yamsakul P, Yano T, Eiamsam-ang T. The Role of Exogenous Non-Starch Polysaccharide Enzymes in Enhancing Digestibility and Performance of Pig. *Biology*. 2026, 15 (1): 1-15. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [48] Chuang WY, Hsieh YC, Lee TT. Evaluation of Waste Mushroom Compost as a Feed Supplement and Its Effects on the Fat Metabolism and Antioxidant Capacity of Broilers. *Animals*. 2020, 10 (3): 1-16. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [49] Fan B, Li M, Shi Z, *et al.* Effects of Enzyme-Microbe Co-Fermented *Ganoderma lucidum* Spent Substrate on Growth Performance, Apparent Nutrient Digestibility, Organ Indices, and Gut Microbiota in Yellow-Feathered Broilers. *Animals*. 2026, 16 (6): 1-13. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [50] Mthana MS, Mthiyane DMN. Low dietary oyster mushroom spent substrate limitedly ameliorates detrimental effects of feeding combined marula seed cake and mucuna seed meal as soya bean replacements in broiler chickens. *Tropical Animal Health and Production*. 2024, 56: 1-17. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [51] Maniraguha V, Hong JS, Yu M, *et al.* Feeding dietary non-starch polysaccharides supplemented with xylanase could improve the performance of broilers. *Journal of Animal Science and Technology*. 2024, 66 (5): 999-1009. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [52] Jiang YX, Li SM, Fan JH, *et al.* Effects of dietary supplementation with *Ganoderma lucidum* residue on apparent nutrient digestibility and emissions of ammonia and hydrogen sulfide in laying hens. *China Poultry*. 2019, 41 (22): 61-63. (in Chinese)
- [53] Huang X, Yao YL, Li C, *et al.* Effects of dietary *Ganoderma lucidum* fungus chaff supplemental level on laying performance, egg quality and serum biochemical indices of laying ducks. *Chinese Journal of Animal Nutrition*. 2020, 32 (8): 3654-3661. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [54] Morshedy SA, Gad KM, Basyony MM, *et al.* The feasibility of partial replacement of berseem hay by spent mushroom (*Pleurotus ostreatus*) substrate in rabbit diets on growth performance, digestibility, caecum fermentation, and economic efficiency. *Archives of Animal Nutrition*. 2023, 77 (6): 421-436. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [55] Van Doan H, Hoseinifar SH, Tapingkae W, *et al.* Effects of *Cordyceps militaris* spent mushroom substrate on mucosal and serum immune parameters, disease resistance and growth performance of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*. 2017, 67: 78-85. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [56] Van Doan H, Hoseinifar SH, Dawood MAO, *et al.* Effects of *Cordyceps militaris* spent mushroom substrate and *Lactobacillus plantarum* on mucosal, serum immunology and growth performance of Nile tilapia (*Oreochromis niloticus*). *Fish & Shellfish Immunology*. 2017, 70: 87-94. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [57] Lee PT, Wu YS, Tseng CC, *et al.* Dietary *Agaricus blazei* Spent Substrate Improves Disease Resistance of Nile Tilapia (*Oreochromis niloticus*) Against *Streptococcus agalactiae* in Vivo. *Journal of Marine Science and Engineering*. 2022, 10 (1): 1-17. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [58] Zakaria Z, Abd Rasib NA, Tompong MF. Spent Mushroom Substrate Based Fish Feed Affects the Growth of Catfish (*Clarias gariepinus*). *IOP Conference Series: Earth and Environmental Science*. 2021, 765: 1-10. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [59] Eggink KM, Pedersen PB, Lund I, *et al.* Chitin digestibility and intestinal exochitinase activity in Nile tilapia and rainbow trout fed different black soldier fly larvae meal size fractions. *Aquaculture Research*. 2022, 53 (16): 5536-5546. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [60] Kanjanarat K, Laksanawimol P, Lersawhanwaree J, *et al.* Utilizing spent mushroom substrate for rearing black soldier fly (*Hermetia illucens*) larvae: Enhancing fertilizer efficiency and improving animal feed quality for sustainable agriculture. *PeerJ*. 2025, 13: 1-20. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [61] Liu Y, Wu Q, Wu X, *et al.* Structure, preparation, modification, and bioactivities of beta-glucan and mannan from yeast cell wall: A review. *International Journal of Biological Macromolecules*. 2021, 173: 445-456. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [62] Qiu Q, Li Y, Zhan Z, *et al.* Genomic and Pangenomic Analysis of *Khuyveromyces Marxianus* SHY2, a Novel Strain with High Proteolytic Potential. *Biofeed Science and Technology*. 2026, 1 (1): 1-7. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [63] Wang D, Zhang M, Wan J, *et al.* Enhancing digestibility and intestinal peptide release of *Pleurotus eryngii* protein: An enzymatic approach. *Journal of Fungi*. 2024, 10: 1-17. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [64] Zhang A, He W, Han Y, Zheng A, Chen Z, *et al.* Cooperative Fermentation Using Multiple Microorganisms and Enzymes Potentially Enhances the Nutritional Value of Spent Mushroom Substrate. *Agriculture*. 2024, 14 (4): 1-14. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [65] Long J, Wang X, Qiu S, *et al.* Construction of cellulose-degrading microbial consortium and evaluation of their ability to degrade spent mushroom substrate. *Frontiers in Microbiology*. 2024, 15: 1-14. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [66] Liu B, Li N, Chen F, Zhang J, *et al.* Review on the release mechanism and debittering technology of bitter peptides from protein hydrolysates. *Comprehensive Reviews in Food Science and Food Safety*. 2022, 21 (6): 5153-5170. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [67] Jia T, Yun Y, Yu Z. Propionic Acid and Sodium Benzoate Affected Biogenic Amine Formation, Microbial Community, and Quality of Qat Silage. *Frontiers in Microbiology*. 2021, 12: 1-16. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [68] Wang Q, Qi Z, Fu W, *et al.* Research and Prospects of Enzymatic Hydrolysis and Microbial Fermentation Technologies in Protein Raw Materials for Aquatic Feed. *Fermentation*. 2024, 10 (12): 1-21. [\[Google Scholar\]](#) [\[CrossRef\]](#)

- [69] Pina-Martín S, Naranjo Fernández E, Orts Gómez JM, *et al.* A novel sequential biphasic bioprocess (enzymatic/fermentative) for the valorisation of orange peel waste into products with potential biostimulant and biofertilizer activity. *Biomass Conversion and Biorefinery*. 2026, 16: 1-12. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [70] Ab Rhaman SMS, Naher L, Siddiquee S. Mushroom Quality Related with Various Substrates' Bioaccumulation and Translocation of Heavy Metals. *Journal of Fungi*. 2022, 8 (1): 1-17. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [71] INRAE, CIRAD, AFZ. Tables of composition and nutritional values of feed materials for ruminants, pigs, poultry, rabbits, horses and salmonids. Retrieved. Available online: <https://www.feedtables.com/> (accessed on 8 August 2026)
- [72] Percie du Sert N, Hurst V, Ahluwalia A, *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biology*. 2020, 18 (7): 1-65. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [73] Fernando LD, Zhao W, Gautam I, *et al.* Polysaccharide assemblies in fungal and plant cell walls explored by solid-state NMR. *Structure*. 2023, 31 (11): 1375-1385. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [74] Nussbaum N, Balmelli L, Steiger N, *et al.* Quantifying fungal growth in 3D: An ergosterol-based method to distinguish growth modes. *Materials Advances*. 2025, 6 (20): 7261-7272. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [75] Nguyen HT, Bedford MR, Wu SB, *et al.* Soluble non-starch polysaccharide modulates broiler gastrointestinal tract environment. *Poultry Science*. 2021, 100 (8): 101183. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [76] Zou G, Li T, Mijakovic I, *et al.* Synthetic biology enables mushrooms to meet emerging sustainable challenges. *Frontiers in Microbiology*. 2024, 15: 1-5. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [77] Roura E, Tedo G, Ipharraguerre IR. Use of double-choice feeding to quantify feed ingredient preferences in pigs. *Livestock Science*. 2009, 123 (2-3): 129-137. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [78] Carroll AL, Kononoff PJ. Feed preference and feed intake: Is there a real-world link that NASEM predictive models overlook? WCDS Advances in Dairy Technology, the Red Deer Resort & Casino, Canada, March 5 to 8, 2024, 75-80.
- [79] Seo S, Jeon S, Ha JK. Guidelines for experimental design and statistical analyses in animal studies submitted for publication in the Asian-Australasian Journal of Animal Sciences. *Asian-Australasian Journal of Animal Sciences*. 2018, 31 (9): 1381-1386. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [80] Dwan K, Gamble C, Williamson PR, *et al.* Systematic review of the empirical evidence of study publication bias and outcome reporting bias: An updated review. *PLoS One*. 2008, 3 (8): 1-31. [\[Google Scholar\]](#) [\[CrossRef\]](#)
- [81] Han B, Baruah K, Cox E, *et al.* Structure-functional activity relationship of beta-glucans from the perspective of immunomodulation: A mini-review. *Frontiers in Immunology*. 2020, 11: 1-8. [\[Google Scholar\]](#) [\[CrossRef\]](#)