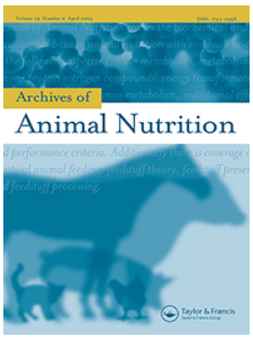

Archives of Animal Nutrition

ISSN: 1745-039X (Print) 1477-2817 (Online) Journal homepage: www.tandfonline.com/journals/gaan20

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**To cite this article:** Robert Ringseis & Klaus Eder (2025) Up to date evidence about the suitability of filamentous fungi as a feed source for broilers, Archives of Animal Nutrition, 79:2, 73-94, DOI: 10.1080/1745039X.2025.2495619

**To link to this article:** https://doi.org/10.1080/1745039X.2025.2495619

ARCHIVES OF ANIMAL NUTRITION
2025, VOL. 79, NO. 2, 73–94
https://doi.org/10.1080/1745039X.2025.2495619

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# Up to date evidence about the suitability of filamentous fungi as a feed source for broilers

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**ABSTRACT**

Biomass from cultivated edible mushrooms can be sustainably produced using residual lignocellulosic sidestreams from agri-industrial and wood processing and is a rich source of a variety of bioactive compounds. While edible mushrooms have a long history as a tasty and healthy food for humans, the potential of mushrooms (fungal fruiting bodies), fungal mycelia and by-products from mushroom production (stem residues, spent mushroom substrate) as feed component for poultry is less acknowledged. Based on this, the present review aims to describe the role of filamentous fungi in circular feed production, characterise the nutritional value of fungal biomass, and provide up to date evidence about the efficacy of feeding fungal biomass on performance of broilers. Feeding studies with healthy and parasite-infected broilers using fungal fruiting bodies, mycelia, stem residues or spent mushroom substrate nearly equally showed either positive or neutral effects on growth performance, whereas impairments of performance and carcass parameters were not reported. Improvements of broiler performance in response to feeding fungal biomass were at least partially associated with a beneficial modulation of the gut microbiota community structure – effects that are most likely attributed to the presence of fermentable polysaccharides and phenolic compounds with prebiotic and selective antimicrobial activities, respectively. Based on the evidence from literature presented in this review, the inclusion of fungal biomass, particularly inexpensive by-products from mushroom production like stem residues and spent mushroom substrate, in broiler diets can be recommended, because even if no growth-promoting effects are induced the partial replacement of less sustainably produced feed components by fungal biomass improves the environmental impact of broiler production. Considering that the number of broiler studies showing either positive or neutral effects of fungal biomass on growth performance is

**ARTICLE HISTORY**
Received 24 January 2025
Accepted 15 April 2025

**KEYWORDS**
Broilers; fungal biomass; mushrooms; sustainability; performance

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/1745039X.2025.2495619

broadly balanced and divergent study outcomes are reported even for biomass from the same fungal species, future studies are necessary to identify the specific requirements of fungal biomass responsible for promoting broiler performance.

## 1. Introduction

The provision of sufficient amounts of adequate feed sources for farm animals is an increasing global challenge. Considering the steadily growing world population, which is predicted to reach 9.7 billion by 2050 (FAO 2018), and the associated increase in the consumption of animal products, especially meat, the demand for feed is markedly rising. Based on the latest OECD-FAO agricultural outlook, global meat production is projected to rise by 12% from 2024 to 2033, with poultry meat accounting for 50% of the additional meat produced (OECD/FAO 2024). The strong increase of global production of poultry meat is largely attributed to its relatively low production costs making poultry meat more affordable for the general population compared with other meat sources. The global need for increasing feed production is complicated by the fact that natural resources are becoming increasingly scarce due to different reasons including climate change, water shortage, land degradation, urbanisation and ongoing food-feed competition. In light of this challenging scenario, future feeding concepts for poultry must ensure that the feed components are made available in a sustainable, environmental-friendly and resource-efficient manner and food-feed competition is low.

Agri-industrial by-products resulting from processing of grain crops, legumes or oil seeds represent a large biomass resource, which is increasingly available due to the worldwide expansion of agricultural production driven by the growing world population. Utilizing this regionally available biomass as feed resource increases sustainability of animal production, because no additional resources like arable land, water or fossil fuels are required for feed production. However, for poultry this biomass can only be used as feed to a limited extent due to the high content of plant cell wall components (e.g. lignin and cellulose), which impair nutrient and energy utilisation. One strategy to make this lignocellulosic biomass usable as feed for poultry is its valorisation by filamentous fungi due to their unique ability to convert low-value, lignocellulosic biomass into edible fungal biomass (Wikandari et al. 2022). Filamentous fungi are multicellular (unlike unicellular yeasts) eukaryotic microorganisms, which consist of thread-like, filamentous cells, called hypha, that form a network of interconnected hyphal threads referred to as mycelium (Cole 1996). Fruiting bodies, also called sporocarps, with the characteristic cap (pileus) and stem (stipe) are formed at the surface from the mycelium as reproductive structures to generate sexual spores. The fruiting bodies of Basidiomycetes, to which many edible fungi belong, are colloquially called mushrooms (Cohen et al. 2014). While edible mushrooms have a long history as a tasty and healthy food for humans, the potential of mushrooms and their by-products (stem residues, spent mushroom substrate (SMS)) as feed component for poultry is less acknowledged. Based on this, the present review aims to 1) describe the role of filamentous fungi in circular feed production, 2) characterise the nutritional value of fungal biomass, and 3) provide up to date evidence about

the efficacy of feeding fungal biomass on performance of broilers being the most important meat-producing poultry species.

## 2. Usefulness of filamentous fungi in circular feed production

Filamentous fungi, to which commercially cultivated edible mushrooms of the genera *Agaricus (A.)*, *Auricularia*, *Flammulina (F.)*, *Ganoderma (G.)*, *Hericium (H.)*, *Lentinus (L.)*, *Pleurotus (P.)*, and *Tremella (T.)* belong, can be perfectly integrated into circular feed production systems (Meyer et al. 2020). Most filamentous fungi are saprophytic meaning that the carbon source being used as energy source for growth is derived from non-living organic substrates, such as dead plants and animals. In order to use these substrates as a carbon source, filamentous fungi produce and secrete a large variety of extracellular enzymes for the hydrolysis of carbohydrates, proteins and lipids, that degrade the substrate into smaller units, e.g. monosaccharides and oligosaccharides, being taken up into the hyphae (Cole 1996). Due to the secretion of cellulases, xylanases, mannases, arabinases and a battery of ligninolytic enzymes like lignin peroxidases, manganese peroxidases and laccases, which work synergistically to fully degrade even complex polymers of the plant cell wall, fungal species belonging to Basidiomycetes have the particular ability of efficiently converting low-value lignocellulosic sidestreams, such as agri-industrial by-products and even products from forestry including forest residues, short rotation coppices and by-products from wood processing, such as sawdust and wood chips, into high-value mycelial biomass which can be used as feed. As a side-effect, biomass resource efficiency is increased and waste accumulation is avoided. Despite these beneficial aspects of the integration of fungi into circular feed production, a factor limiting the sustainability of fungal biomass as feed is the necessity to perform an energy-intensive drying step prior to including the fungal biomass into a compound feed.

## 3. Most important production techniques for fungal biomass and by-products from mushroom production

Generally, production of fungal biomass is a prime example for an efficient utilisation of residual lignocellulosic biomass instead of disposing this biomass unexploited as waste. Compared to the cultivation of other agricultural crops like grains or legumes, production of fungal biomass requires substantially less space due to vertical arrangement of the cultivation areas. In addition, given that mushroom cultivation occurs indoors, mushroom production is largely independent from the outdoor climate and can be carried out over the whole year. Commercial production of fruiting bodies of edible mushroom species, such as white button mushroom (also known as Champignon, *Agaricus bisporus*), oyster mushroom (*P. ostreatus*), shiitake mushroom (*L. edodes*) and enoki mushroom (*F. velutipes*), occurs in a production process called solid-state fermentation. While the typical cultivation substrate is based on lignocellulosic materials like compost, straw or sawdust (Sánchez 2010), the specific composition and physical structure of the substrate varies depending on the fungal species. Critical factors affecting the yield of fruiting bodies are the ratio in the concentrations of carbon and nitrogen and the pH value of the mushroom substrate (Carrasco et al. 2018). Apart from the mushroom

fruiting bodies, which are usually marketed for consumption as a food, mushroom stipes and poor quality mushrooms not meeting commercial standards, together making up 5–20% of the mushroom yield (Antunes et al. 2020), can be used for feeding purposes. The main by-product from solid-state fermentation being left after harvesting the fruiting bodies is the SMS, from which approximately 3–5 kg is generated per kg fresh mushrooms depending on the substrate, the production system, and the cultivated species (Zisopoulos et al. 2016). Due to the large amounts generated, efficient recycling of this residual biomass is crucial for sustainable mushroom production. In this regard, utilisation of SMS as an inexpensive and sustainable feed source is of great interest.

An alternative method for the production of fungal mycelia without producing mushrooms is submerged (liquid) fermentation in a bioreactor, in which the fungus is cultivated in different liquid substrates under controlled conditions (temperature, aeration, pH), and from which the fungal mycelium is finally collected by filtration (Antunes et al. 2020). As substrates for submerged fermentation different agri-industrial sidestreams come into question. For instance, isomaltulose molasses – a side stream of the sugar industry – has been used as the sole carbon source for the production of mycelial biomass of *P. sajor-caju via* submerged fermentation in a recent study (Maheshwari et al. 2020). The clear advantages over solid state fermentation are shorter production cycles, lower requirement for space, better control of contamination and less complicated environmental regimes (Papaspyridi et al. 2012; Scholtmeijer et al. 2023).

## 4. Nutritional composition of fungal fruiting bodies and mycelia and by-products from mushroom production

### 4.1. Fungal fruiting bodies and mycelia

Fresh mushrooms consist mainly of water explaining that their dry matter (DM) content is very low ranging between 90 and 200 g/kg fresh matter for commercially important mushroom species (Table 1). As shown in Table 1, the main part of the DM is constituted of carbohydrates accounting for 715–870 g/kg DM in *A. bisporus*, *F. velutipes*, *L. edodes*, *P. ostreatus* and *P. eryngii* (Reis et al. 2012). The mushroom carbohydrates consist of low molecular weight carbohydrates and storage and structural polysaccharides. Mannitol, mannose and trehalose are the main representatives of low molecular weight carbohydrates in the above mushroom species being in the range of 28–39 g/kg DM (Kalač 2013). The main storage polysaccharides found in fungal mycelia of cultivated mushrooms is the

**Table 1.** Nutritional composition of fruiting bodies of commercially important mushroom species according to Reis et al. (2012).

| Mushroom species | Local/English name | Moisture | Carbohydrates | Crude protein | Lipids | Ash |
|---|---|---|---|---|---|---|
| | | [g/kg FM] | | [g/kg DM] | | |
| *A. bisporus* [white] | Champignon | 913 | 740 | 141 | 22 | 97 |
| *A. bisporus* [brown] | Portobello | 916 | 715 | 154 | 17 | 114 |
| *P. eryngii* | King oyster | 890 | 814 | 110 | 15 | 62 |
| *P. ostreatus* | Oyster | 892 | 859 | 70 | 14 | 57 |
| *L. edodes* | Shiitake | 798 | 871 | 44 | 17 | 67 |
| *F. velutipes* | Enoki | 879 | 871 | 39 | 17 | 73 |

Abbreviations: DM, dry matter; FM, fresh matter.

α-glucan glycogen, whose content ranges between 10 and 39 g/kg DM (Sari et al. 2017). Amongst the structural carbohydrates present in the cell wall of filamentous fungi, chitin and β-glucans are the dominating representatives making up 6–100 g/kg DM and 90–250 g/kg DM, respectively, in the fruiting bodies of cultivated mushroom species (Nitschke et al. 2011; Sari et al. 2017). The β-glucans consist mainly of a β-1,3-linked glucose backbone, in which every second or third glucose molecule can be β-1,6-linked with another β-1,3-linked glucose chain (Nitschke et al. 2011). Grifolan, pleuran and lentinan are frequently occurring prototypes of branched β-1,3–1,6-glucans in cultivated mushroom species (Chakraborty et al. 2020). The content of β-glucans in fruiting bodies is significantly higher in the stipes than in the caps (Sari et al. 2017). The latter is of relevance for commercially cultivated mushrooms considering that the stipe of cultured mushrooms is cut off either completely or partially after harvest. Thus, mushroom stipes as by-products from commercial mushroom production can be used as inexpensive dietary sources for β-glucans. In addition, the β-glucan content in many cultivated mushroom species is higher in the fruiting bodies than in the mycelia from submerged fermentation (Nitschke et al. 2011). This has been attributed to the fact that the major function of β-glucans, together with chitin, is to stabilise the fungal cell wall, and the fruiting body, which is growing outside the substrate has to withstand more physical forces, than the mycelium growing inside the substrate.

Fungal fruiting bodies and mycelia are also a significant source of protein. Based on a nitrogen-to-protein conversion factor of 4.38, which considers the high amount of non-protein nitrogen in fungal biomass, the protein content in the most important cultivated mushroom species is in the range of 40–160 g/kg DM (Table 1). Within- and between-species variations in protein content are often explained by the nitrogen content of the substrate, with an increase in of the carbon:nitrogen-ratio reported to be correlated to a decrease in fungal protein content (Hoa et al. 2015). Like the content of structural polysaccharides, the content of protein differs depending on the morphological part of the mushroom, with higher protein concentrations found in the cap than in the stipe (Ayimbila and Keawsompong 2023). The protein quality of some important commercial mushrooms can be regarded as high considering an essential amino acid score of greater than 1 reported for *A. bisporus*, *P. ostreatus* and *L. edodes* (González et al. 2020). Protein digestibility of different mushrooms as assessed in monogastric animals was found to vary greatly between different mushroom species (43–80%), and to be rather lower in some cases (e.g. 43% for *P. sajor-caju*; González et al. 2020). Differences in the content of indigestible polysaccharides across mushroom species might account for these variations in protein digestibility, because these structural polysaccharides are known to reduce nutrient digestibility.

The lipid content in mushrooms is generally very low making up approximately 10–20 g/kg DM in the most important cultivated mushrooms (Kalač 2013, Table 1). The majority (approx. 75%) of total lipids in these mushrooms are polyunsaturated (linoleic acid, 48.6% of total lipids; α-linolenic acid, 1.3% of total lipids) and monounsaturated (oleic acid, 22% of total lipids) fatty acids, whereas 25% are saturated fatty acids, mainly palmitic and stearic acid. Few reports show differences in the fatty acid composition of different morphological parts of the fruiting body. For example, *Boletus edulis*

**Table 2.** Reported concentration ranges of minerals in cultivated mushrooms according to Kalač (2013).

| Bulk elements | Concentration range [g/kg DM] | Trace elements | Concentration range [mg/kg DM] |
|---|---|---|---|
| Calcium | 0.1–0.5 | Copper | 20–100 |
| Chlorine | 1–6 | Iodine | 0.07–0.5 |
| Magnesium | 0.8–1.8 | Iron | 50–300 |
| Phosphorus | 5–10 | Manganese | 10–60 |
| Potassium | 20–40 | Selenium | <2–20 |
| Sodium | 0.1–0.4 | Zinc | 25–200 |
| Sulfur | 1–6 | | |

Abbreviations: DM, dry matter.

mushrooms were found to contain 75% polyunsaturated fatty acids of total lipids in the caps but only 55% in the stipes (Pietrzak-Fiećko 2016).

Crude ash content of cultivated edible mushrooms is in the range of 60–110 g/kg DM (Table 1). The minerals contributing most to the ash content of cultivated mushrooms are shown in Table 2. A large number of papers exist reporting trace element contents in wild-growing mushrooms, but the scientific interest regarding trace elements is mainly focused on detrimental trace elements, such as cadmium, mercury, arsenic or lead, and radioactive elements due to the strong bioaccumulation capability of many mushroom species. In contrast, data about trace element contents in cultivated mushrooms is scarce. An impression about the mean contents of essential trace elements in fruiting bodies can be obtained from wild-growing mushrooms from areas which are not polluted by toxic or radioactive elements (Table 2).

Cultivated mushrooms are also a significant source of different vitamins including the B-vitamins thiamine, riboflavine, pyrodoxine, biotin, folic acid, and niacin as well as vitamin C (Cağlarirmak 2011). In addition, vitamin E and β-carotene, a potent provitamin A and antioxidant, are found in cultivated mushrooms, which together with vitamin C contribute to antioxidant effects of mushroom consumption (Ferreira et al. 2009). Moreover, mushrooms are a notable source of vitamin $D_2$ (Cardwell et al. 2018), whose content can be considerably increased by exposure to ultraviolet irradiation due to formation from ergosterol. For instance, UVB irradiation elevated the vitamin $D_2$ content from 3.1 to 37 mg/kg DM and from 0.3 to 57 mg/kg DW in *P. ostreatus* and *A. bisporus* fruiting bodies, respectively (Gallotti and Lavelli 2020). Similar vitamin $D_2$ contents were reported in another study for UVB-irradiated fruiting bodies from *L. edodes* (26 mg/kg DM), *A. bisporus* (32 mg/kg DM) and *P. ostreatus* (29 mg/kg DM) (Malik et al. 2022).

### 4.2. Spent mushroom substrate (SMS)

The SMS is generated during mushroom cultivation (solid-state fermentation) as a result of enzymatic degradation of the substrate raw materials, such as lignocellulosic by-products from forestry, agriculture and agri-industrial processing, and incorporation of the released nutrients and other substrate nutrients into the fungal biomass. Since lignin, cellulose and hemicelluloses are not completely degraded by the fungi [mass losses have been reported to be 26–75% for *L. edodes* and several *P. spp*. (Gong et al. 2012; Chen et al. 2022)], residual, not degraded plant cell-wall components and the fungal mycelium are

the main constituents of the SMS. Thus, the partial degradation of plant cell-wall components from the substrate raw materials along with the growth of the fungal mycelium, which is rich in structural carbohydrates (β-glucans, chitin), proteins and many bioactive compounds as well as certain micronutrients (see Chapter 3.1), causes a significant valorisation of the poorly digestible, "low-value" cultivation substrate to a "high-value"product, the SMS. Despite that this valorization process is characteristic for all SMS, it is comprehensible that the composition of specific SMS varies dependent on the cultivation substrate and the mushroom species. Thus, the nutritional value of SMS is exemplified for solid-state fermentation of *P. ostreatus* in faba bean hulls as reported in the study from Ivarsson et al. (2021). In this study, solid-state fermentation of *P. ostreatus* was accompanied by an increase of the protein content from about 200 g/kg DM in the cultivation substrate (faba bean hulls) to approx. 350 g/kg diet in the SMS. In addition, the content of ash and neutral lipids increased from 29.5 and 13.1 g/kg DM, respectively, in the initial cultivation substrate to 57.1 and 31.0 g/kg DM, respectively, in the SMS. In agreement with the increase of ash content in the SMS, the concentrations of several essential minerals including calcium, potassium, magnesium, sodium, iron, cobalt, manganese and zinc increased in the SMS compared with the initial substrate raw materials. In contrast, the content of different anti-nutritional compounds, such as condensed tannins, vicine and convicine, was significantly lower in the SMS than in the substrate raw materials used for cultivation of *P. ostreatus* (Ivarsson et al. 2021), indicating that the digestibility of the SMS was improved by this valorisation process. Collectively, SMS is a sustainable and nutrient-rich by-product from mushroom cultivation which has potential as a feed source for farm animals.

## 5. Non-nutritive bioactive compounds in fungal biomass

Apart from the above-described nutritive compounds, such as carbohydrates, polyunsaturated fatty acids, major and trace elements or vitamins, mushrooms are also known to contain several non-nutritive compounds. These display a wide array of different health-promoting activities, such as antimicrobial, antiinflammatory, antioxidant, immunomodulatory, antitumor, hypolipidemic and hypotensive effects, explaining that certain mushrooms have also long been used for medicinal purposes (e.g. medicinal mushrooms like shiitake and maitake). Amongst these compounds, ergothioneine – a sulphur-containing amino acid derivative – is quantitatively important. Own analyses revealed a concentration of 0.84 g/kg DM for ergothioneine in *P. sajor-caju* mycelium produced from submerged fermentation (Maheshwari et al. 2020). A similar concentration for ergothioneine was demonstrated for *P. citrinopileatus* fruiting bodies (Tsiantas et al. 2021). Higher concentrations of ergothioneine were found in *P. ostreatus* fruiting bodies (2.4 g/kg DM; Cohen et al. 2014), whereas lower concentrations were documented for fruiting bodies of *P. spp*. in a study from Woldegiorgis et al. (2014) and for C. *militaris* in studies from Jędrejko et al. (2022) and Cohen et al. (2014). Ergothioneine is known to possess both, antioxidant and antiinflammatory activities (Rahman et al. 2003). In addition, cordycepin, the trivial name for the adenosine nucleoside 3′-deoxyadenosine, is a bioactive compound with antioxidant properties found both in free-form and bound to a glycoside residue in *Cordyceps spp*. Its concentration was reported to be in the range of 0.25–0.57 g/kg DM in fruiting bodies from *C. militaris* (Jędrejko et al. 2022). Besides

antioxidant activities, antiinflammatory, antiviral, and even antitumor activities have been attributed to cordycepin (Radhi et al. 2021). Furthermore, phenolic compounds are present in different mushrooms, like *p*-hydroxybenzoic acid, gallic acid, vanillic acid, caffeic acid, ferulic acid, quercitrin and rutoside. In fruiting bodies from *C. militaris*, the phenolic compound with the highest concentration was quercitrin making up 0.02 g/kg DM (Jędrejko et al. 2022). Documented concentrations for *p*-hydroxybenzoic acid were 0.04 and 0.002 g/kg DM for fruiting bodies from *P. eryngii* and *P. citrinopileatus*, respectively (Krakowska et al. 2020). The concentration of total phenolic compounds as assessed by gallic acid equivalents was 2.74 and 3.32 g/kg DM for *P. ostreatus* and *P. cornucopiae* (Kolayli et al. 2012). Like cordycepin, phenolic compounds are well-known for their antioxidant properties but their effects also include antiinflammatory, immunomodulatory, antimicrobial and many other activities. Interestingly, mushrooms were also demonstrated to contain the cholesterol-lowering compound lovastatin. Reported lovastatin concentrations show a large variation from 0.01 g/kg DM for fruiting bodies from *A. bisporus* (Tsiantas et al. 2021), *H. erinaceus*, and *G. lucidum* (Cohen et al. 2014), 0.1 g/kg DM for *P. eryngii* (Chen et al. 2012), 0.3 g/kg DM for *C. militaris* (Jędrejko et al. 2022), and 0.6 g/kg DM for *P. ostreatus* (Chen et al. 2012). Cholesterol-lowering effects might be also explained by the presence of ergosterol (Gil-Ramírez et al. 2014), which reduces cholesterol absorption due to competition with cholesterol for incorporation into mixed micelles in the intestine. Concentrations of free ergosterol in fruiting bodies were found to be between 3.3 and 6.4 g/kg DM for *A. bisporus*, *B. edulis*, *L. edodes* and *P. ostreatus* (Villares et al. 2014). The non-proteogenic amino acid γ-aminobutyric acid is a further bioactive compound found in many edible mushrooms which is known to cause hypotensive effects (Rauf et al. 2023). Furthermore, different mushroom species including some of the commercially used ones (*L. edodes*, *A. bisporus*) and *C. militaris* have been shown to contain several bioactive peptides – peptide fragments released during digestion which are absorbed in intact form – with different biological activities, such as immunomodulatory, antihypertensive, anticarcinogenic, hepatoprotective and antimicrobial (González et al. 2020; Ayimbila and Keawsompong 2023). Despite that mushrooms contain several more non-nutritive bioactive compounds, the above-described examples demonstrate that mushrooms are a rich source of a large variety of bioactive compounds which contribute to different metabolic and health-promoting effects of mushroom consumption.

## 6. Effects of fungal products on growth performance of broilers

### 6.1. Effects of fungal mycelia or fungal mycelial extracts

#### 6.1.1. Studies with healthy broilers

Several studies evaluated the efficacy of fungal mycelia or fungal mycelial extracts on growth performance of healthy broilers with different outcomes (Supplementary Table 1). A positive effect of 3-wk supplementation (from d 1 to 21 of age) of the diet with an *Aspergillus niger* mycelium (40 g/kg diet) on performance was observed in a study with Hubbard broilers (Zyła et al. 2000). Broilers fed the diet supplemented with the *A. niger* mycelium had a higher feed intake and BW gain and a lower feed: gain ratio than broilers fed the unsupplemented diet. An additional group of broilers

fed a diet with 40 g/kg diet of the *A. niger* mycelium and 1,300 U phytase showed a similar performance as the group of broilers fed the diet supplemented with *A. niger* mycelium only. A further observation from this study was that dietary inclusion of the fungal mycelium increased the bursa of Fabricius weight compared to feeding the unsupplemented diet. Considering that this lymphoid tissue plays an important defensive role against pathogens at the gut mucosa, the growth-promoting effect of the *A. niger* mycelium might be attributed to its protective effect against gut pathogens. Also, Giannenas et al. (2010) showed that female Ross 308 broilers supplemented with 20 g/kg diet of *A. bisporus* mushroom from d 1 to 42 of age had a better growth performance (higher final BW and BW gain) and an improved feed efficiency compared to broilers fed the unsupplemented diet. In addition, an increase of the antioxidative capacity of tissues (liver, breast muscle, thigh muscle) was found in the broilers fed the *A. bisporus*-supplemented diet. An improved antioxidative capacity of tissues might has contributed to a better growth performance, because tissues are better protected against the harmful effects of high levels of reactive oxygen species which can also trigger inflammatory processes. Inflammatory processes are also detrimental to growing farm animals as the inflammatory process consumes energy and building substrates, such as glucose and amino acids, which are consequently less available for BW gain (Gessner et al. 2017). In addition, indications for a prebiotic effect of the *A. bisporus* mushroom were provided in another report from Giannenas et al. (2010). In the ileum and the caecum, the well-known gut protective *Lactobacillus spp*. and *Bifidobacteria* population was higher and the ratio of *E. coli* to *Lactobacillus spp*. was lower in the broilers supplemented with 20 g/kg diet of *A. bisporus* compared to the non-supplemented broilers. Considering the well-documented prebiotic effects of β-glucans and chitin, both of which are contained in mushrooms including *A. bisporus*, the above-described changes in the gut microbial community structure are likely caused by the presence of these prebiotic polysaccharides. Indeed, broiler studies dealing with the dietary supplementation of isolated β-glucans or chitin oligosaccharides revealed similar shifts in the gut microbial community structure with an increased abundance of gut barrier-protective commensals and a decreased abundance of obligate pathogens, such as *E. coli* (Zhang et al. 2020; Khan et al. 2022) – effects which are typically accompanied by an improved gut function and nutrient digestion. In line with this, evidence has been gained that dietary supplementation with β-glucans increases energy digestibility in broilers (Zhang et al. 2020). In addition, evidence being indicative for an improvement of gut function has been provided from a broiler study in which dietary supplementation of β-glucans at 100 mg/kg diet increased the villus height:crypt depth ratio in the duodenum, the jejunum and the ileum (Amer et al. 2022).

Positive effects on BW gain and caecum microbiota composition (decreased counts of *E. coli*, increased counts of Lactobacilli and Bifidobacteria) were also reported in response to feeding *H. caput-medusae* polysaccharides to female Arbor Acres broilers from d 1 to 42 of age (Shang et al. 2015). Increased numbers of *Lactobacillus spp*. and *Bifidobacterium spp*. as well as reduced numbers of Coliforms in the gut of broilers have been also demonstrated in response to feeding other fermentable carbohydrates (Rehman et al. 2007, 2008), and are indicative of a more favourable intestinal microbiota composition which goes along with improved gastrointestinal function

and animal performance. Beyond fermentable carbohydrates, phenolic compounds which exhibit a selective antimicrobial activity might have also contributed to the observed change of the gut microbiota composition. According to the study of Giannenas et al. (2010) the concentration of gallic acid equivalents representing total phenolic compounds in the mushroom was 8.85 g/kg DM. An improvement of performance over the whole period (d 1–42 of age) was reported also from a 6-wk-feeding trial with Cobb broilers in which a maize-soybean meal mixture colonised by *P. ostreatus* was included into a standard broiler diet at different levels (5, 10, 100 and 200 g/kg diet) (Santos et al. 2015). While feed conversion was improved already at inclusion levels of ≥5 g/kg diet, feed intake and BW gain were increased only at inclusion levels of ≥10 g/kg diet. Likewise, feeding diets with only 0.4 (d 1–21 of age) and 0.3 (d 22–42 of age) g/kg diet of a fungal mycelium from *Scytalidium (S.) acidophilum* increased feed intake and BW gain during the whole period in male Cobb broilers (Huang et al. 2004) clearly indicating a performance promoting effect of this fungal mycelium. Although the authors speculated that the positive effect of *S. acidophilum* mycelium was mediated by a prebiotic effect, the authors did not provide appropriate data, such as microbial community composition in the gut of the broilers, supporting this assumption. In addition, an effect of *S. acidophilum* mycelium on antigen-induced production of antibodies (IgA, IgG) could be excluded as a mechanism explaining the performance promoting effect of this fungal mycelium (Huang et al. 2004).

In contrast to feeding *A. bisporus* mushroom, feeding a biotechnologically produced *P. sapidus* mycelium at two different levels (25 and 50 g/kg diet) to Cobb 500 broilers from d 1 to 35 of age did not affect growth performance and nutrient digestibility (Schäfer et al. 2024a). Despite its high content of β-glucans (31% of DM) and chitin (5.3% of DM), feeding the *P. sapidus* mycelium caused no prebiotic effect as indicated by a largely unaltered caecal microbiota community structure, unaltered caecal concentrations of short-chain fatty acids, and a lack of effect on parameters of caecum barrier integrity. In addition, liver transcriptomics and plasma metabolomics revealed only weak or no effects at all, respectively, indicating that the *P. sapidus* mycelium has neither positive nor negative effects on broilers´ performance and metabolism. In addition, gene and protein expression analyses showed that feeding the *P. sapidus* mycelium does not influence main protein anabolic or protein catabolic pathways in the breast muscle of these broilers (Schäfer et al. 2024b) confirming that this fungal mycelium does not affect intermediary metabolism of broilers. No growth-promoting effect of similar doses (10, 30 and 50 g/kg diet) of a fungal mycelium (*F. velutipes*) was also reported from a study with HanHyup broilers (Lee et al. 2012b). However, this study reported a decrease in the count of *Salmonella* bacteria in the caecum of the broilers fed the *F. velutipes* mycelium indicating at least a positive effect on the caecal microbiota composition. Also, no effect of feeding diets with different doses (0.4, 0.8, 1.2, 1.6 and 2.0 g/kg diet) of *A. brasiliensis* mushroom from d 1 to 42 of age on performance parameters (feed intake, BW gain, feed: gain ratio) when compared to feeding the unsupplemented diet was reported from a study with male Ross 308 broilers (Guimarães et al. 2014). In addition, breast meat quality was not compromised by feeding the *A. brasiliensis*-supplemented diets indicating that inclusion of this mushroom into the diet has no benefit for growth performance and does not impair meat quality. Moreover, no effect of feeding a diet with 10 g/kg diet

*A. bisporus* mushroom powder from d 1 to 42 of age on BW gain, feed intake and feed: gain ratio of male Ross 308 broilers was reported from Aziz-Aliabadi et al. (2024). Furthermore, no effect of an aqueous *L. edodes* extract administered *via* the drinking water on BW gain, feed intake and feed efficiency was found in two trials with male and female Ross broilers fed a nutrient-adequate diet (Willis et al. 2007). Despite the lack of effect of the mushroom extract on the growth performance, an increase in the number of faecal *Bifidobacteria*, which are commensal bacteria strengthening the gut barrier, was observed indicating at least a beneficial effect on the gut microbiota composition. Thus, the benefit of administering the above aqueous fungal extract to broilers might be the prevention from colonisation by pathogenic bacteria due to competition with gut barrier protective commensal bacteria.

While the above studies investigated the effect of the whole fungal mycelium, other studies explored the effect of different fungal extracts in which specific fractions of the mycelium were enriched by an extraction step (Koh et al. 2003; Guo et al. 2004a). For instance, in a study with Arbor Acres broilers, the effect of feeding a diet supplemented with different inclusion levels (0.5, 1, 2, 3 and 4 g/kg diet) of two mushroom (*L. edodes*, *T. fuciformis*) polysaccharide extracts for one wk (from d 7 to 14 of age) in comparison to feeding the unsupplemented diet on growth performance from d 7 to 28 of age was investigated (Guo et al. 2004a). The polysaccharide fractions containing high amounts of the fermentable fungal cell wall structures β-glucans and chitin were prepared from the intact mushrooms by an ethanolic extraction step. The efficacy of the mushroom polysaccharide extracts were also compared with that of an antibiotic (virginiamycin) fed to an additional group of broilers. Although BW gain, feed intake and feed conversion-ratio over the whole period (d 7–28 of age) were not improved in broilers fed the diets supplemented with either mushroom polysaccharide extracts or the antibiotic, BW gain during the supplementation period (d 7–14 of age) and from d 15 to 21 of age was higher in broilers fed the antibiotic and the *L. edodes* polysaccharide extract than in broilers fed the unsupplemented diet. In addition, feed conversion-ratio was improved from d 15 to 21 of age in broilers fed the *L. edodes* polysaccharide extract indicating that this fungal extract improves broiler performance, at least during the grower period. A positive effect of feeding an aqueous fungal (*Cordyceps sinensis*) extract (0.6 g/kg diet) on broiler (male Cobb) performance was also reported from Koh et al. (2003). Feeding the *C. sinensis* extract-supplemented basal diet from d 1 to 35 of age increased BW gain and decreased mortality of the broilers compared to feeding the unsupplemented basal diet. BW gain of the broilers fed the *C. sinensis* extract-supplemented diet did not differ from a positive control group of broilers fed the antibiotic avilamycin (20 mg/kg diet) indicating that the fungal extract is as effective as an antibiotic growth promoter. The study further demonstrated a beneficial effect of the *C. sinensis* extract on the small intestinal microbiota composition of the broilers, namely increased counts of *Lactobacillus spp*. and decreased counts of pathogenic *Salmonella spp*. and *E. coli*. This suggested that the growth-promoting effect of *C. sinensis* extract was mediated by a beneficial modulation of the gut microbiota composition.

### *6.1.2. Studies with artificially or naturally infected broilers*

In a few studies the efficacy of fungal mycelia or fungal mycelial extracts was investigated as alternatives to antibiotic growth promoters in infected broilers (Supplementary

Table 2). In an earlier study, feeding a *T. fuciformis* polysaccharide extract (1 g/kg diet) for 1 wk (d 7–14 of age) increased BW gain and reduced mortality from d 7 to 17 of age and from d 18 to 24 of age in Sanhuang broilers, that were vaccinated with *E. tenella* oocysts at d 4 of age and infected with sporulated *E. tenella* oocysts at d 18 of age, compared to *E. tenella*-vaccinated and -infected broilers (Guo et al. 2005). In line with this, caecal oocyst output was significantly lower in the vaccinated *E. tenella*-infected broilers fed the *T. luciformis* polysaccharide extract than in those fed no fungal extract (Guo et al. 2005). This indicated that *T. luciformis* polysaccharide extract has protective activity against the *E.* parasite causing haemorrhagic caecal coccidiosis. In another study from the same group with female 3-d-old Huangyu broilers (Guo et al. 2004b), which were infected with sporulated *E. tenella* oocysts at d 15 of age, feeding diets with two different mushroom polysaccharide extracts (either *T. fuciformis* or *L. edodes*; each 1 g/kg diet) for 1 wk (from d 8 to 14 of age) caused higher levels of serum- and caecum-specific antibodies (IgA, IgM and/or IgG) and a higher antigen-specific proliferation of splenocytes at d 14 and 21 postinfection than feeding the same diet without the fungal polysaccharide extract. Although BW gain, oocyst output and lesion score were not measured in this study, these findings indicated that mushroom polysaccharide extracts enhance systemic and caecal *E. tenella* antigen-binding antibody production and antiparasitic splenocyte proliferation in infected broilers. These immunomodulatory effects possibly explain the above-described improved growth performance of broilers during infection with *E. tenella*. A positive effect of an aqueous fungal extract derived from freshly cultivated *P. ostreatus* mushrooms on growth performance of infected broilers was also reported in a study from Ademola et al. (2019). In this study, 1-day-old Arbor Acres broilers were naturally infected with field strains of *E. spp*. (*E. tenella*, *E. acervulina*, *E. necatrix*, *E. maxima*, *E. brunetti*) at 28 d of age and subsequently assigned to either a negative control group, a positive control group receiving the antibiotic toltrazuril or six groups gavaged with either 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 g/kg BW of the aqueous *P. ostreatus* extract for a period of five days (d 29–33 of age). Aqueous extracts from *P. spp*. mushrooms are known to contain high concentrations of phenolic compounds with antioxidant and antiinflammatory activity as also confirmed in the present study. Broilers gavaged with 0.3–0.5 g/kg BW of the *P. ostreatus* aqueous extract and broilers treated with the antibiotic showed a higher BW gain from d 28 to 35 of age than the negative control group, and broilers gavaged with 0.4 g/kg BW of the *P. ostreatus* aqueous extract had the highest BW gain. Oocyst output *via* the faeces was reduced in the broilers gavaged with the *P. ostreatus* extract at doses ≥0.3 g/kg BW from treatment d 2 onwards. Reduction of oocyst output was most effective for treatment with toltrazuril which caused parasite clearance already at treatment d 2. While the negative control group showed bloody diarrhea throughout the study, the faeces in the positive control group (treatment with toltrazuril) and the groups gavaged with 0.5 and 0.6 g/kg BW of the *P. ostreatus* extract was normal on d 3, 5 and 4 post treatment start. These findings suggested that an aqueous *P. ostreatus* extract is protective against avian coccidiosis. Beneficial effects, such as reduced faecal oocyst output, improved BW gain and reduced feed:gain ratio, were also observed for 5-d-administration of an aqueous *G. applanatum* extract (1 g/kg BW) to broilers infected with mixed coccidial oocysts of *Eimeria spp*. at d 22 of age compared to broilers which were infected but not treated with the mushroom extract (Ahad et al. 2016). Finally, a study from Ogbe et al. (2009)

demonstrated an increased BW gain and a decreased *E. tenella* oocyst output *via* the faeces in response to providing a *G. lucidum* aqueous extract in the drinking water (0.2 g/ml) from d 49 to 56 of age to Ross broilers infected with sporulated *E. tenella* (Houghton strain) oocysts at d 42 of age. In contrast to the above-mentioned studies, a study from Lima et al. (2021) failed to show a beneficial effect of two different fungal mycelia on growth performance of *E. spp*.-infected broilers. In the latter study, male Cobb broilers were artificially infected with sporulated oocysts from different *E. spp*. (*E. acervulina*, *E. maxima*, *E. tenella*) at 11 d of age. While combined treatment of the *E. spp*.-infected broilers with an antibiotic and coccidiostat (avilamycin + sodium monensin) increased feed intake, BW gain and productive efficiency factor over the whole period (d 1–42 of age), treatment with 2 g/kg diet of either *A. subrufescens* or *P. ostreatus* did not improve feed intake, BW gain and productive efficiency factor of *E. spp*.-infected broilers compared to treatment with no additive (Lima et al. 2021). Collectively, the majority of available studies show that fungal mycelial or fungal extracts have the potential to improve growth performance in parasite-infected broilers but more studies are necessary to substantiate these findings.

## 6.2. Effects of by-products from mushroom cultivation

### 6.2.1. Mushroom stem residues

Like studies dealing with the fruiting bodies, feeding studies dealing with the effect of mushroom stem residues in broilers revealed different outcomes depending on the fungal species and/or the dose tested (Supplementary Table 3). A study from Bormon et al. (2024) tested the effect of supplementation of 0.3 g/kg diet of *P. ostreatus* stem residue on growth performance, carcass traits, meat quality, and selected caecal bacterial counts in Arbor Acres broilers. Feeding the diet supplemented with the *P. ostreatus* stem residue to broilers from d 1 to 35 of age increased daily BW gain and reduced the feed:gain-ratio without increasing feed intake compared to broilers fed the unsupplemented diet. Interestingly, growth performance of broilers fed the *P. ostreatus* stem residue-supplemented diet was even better than that of an additional group of broilers fed the unsupplemented diet with an antibiotic (chlortetracycline). In contrast, carcass traits, such as slaughter weight, dressed weight and weights of important meat cuts (wing, thigh, drumstick), and weights of immune organs (bursa of Fabricius, spleen) were not different between groups. In addition, meat quality was positively affected considering that drip loss of meat after 24 h and 7 d was significantly reduced in broilers fed the mushroom stem residue. Moreover, broilers fed the *P. ostreatus* stem residue-supplemented diet and broilers fed the antibiotic had reduced counts of potential pathogens (*E. coli*, *Salmonella spp*.) and a higher count of beneficial *Lactobacillus spp*. in the caecum compared to broilers fed the unsupplemented diet. This suggests that the fungal stem residue has similar growth-promoting effects as certain fungal mycelia (see chapter above). In another study with male Arbor Acres broilers, a positive effect on growth performance was demonstrated for stem residues from *P. eryngii* (Lee et al. 2012a). In this study, four different doses of the mushroom stem residue (1, 5, 10 and 20 g/kg diet) were fed from d 1 to 35 of age. While feed intake did not differ among broiler groups, broilers fed the diet supplemented with ≥5 g/kg diet of the *P. eryngii* stem residue had higher BW gains and a better feed efficiency than broilers fed the unsupplemented diet. In addition,

a beneficial effect of the *P. eryngii* stem residue was seen on the meat quality as drip loss and water holding capacity of breast and thigh fillets was decreased and increased, respectively, in broilers fed the mushroom stem residue at ≥1 g/kg diet. Moreover, the total antioxidant activity and the activities of antioxidant enzymes (catalase and superoxide dismutase) were elevated and the concentration of the lipid oxidation marker malondialdehyde was reduced in serum, liver, spleen, and fillet tissues of broilers fed the *P. eryngii* stem residue at ≥ 1 or ≥5 g/kg diet compared to broilers fed the unsupplemented diet. These findings indicated that the growth-promoting effect of *P. eryngii* stem residue in broilers was due to improving the capacity of the animals to cope with oxidative stress. Like in the study from Bormon et al. (2024), *P. ostreatus* stem residues exhibited a beneficial effect on broiler performance in a study from Hassan et al. (2020). In this study with Ross 308 broilers two different doses (10 and 20 g/kg diet) of the *P. ostreatus* stem residues were compared with the unsupplemented diet and the unsupplemented diet containing an antibiotic (enramycin) for a feeding period of 42 d. Interestingly, a positive effect on BW gain, feed intake and feed conversion was only observed in broilers fed the lower dose of the *P. ostreatus* stem residues and the broilers fed the antibiotic. In addition, blood concentrations of antibodies (IgM, IgG) were higher and the heterophil:lymphocyte-ratio was lower in broilers fed 10 g/kg diet of the *P. ostreatus* stem residue than in those fed the unsupplemented control diet. Moreover, the concentration of reduced glutathione was higher and the concentration of MDA was lower in liver, thigh and breast following a storage period of 5 d in a refrigerator in broilers fed the diet with 10 g/kg diet of the *P. ostreatus* stem residues than in those fed the unsupplemented diet. Collectively, these data show that feeding oyster mushroom waste to broilers has a positive impact on performance and humoral immune response and reduces lipid oxidation of meat during storage.

In contrast, performance parameters (feed intake, daily BW gain, feed conversion) were not improved by feeding diets containing 10 or 20 g/kg diet of *F. velutipes* stem residues to male Arbor Acres broilers from d 1 to 42 of age (Mahfuz et al. 2019). However, feeding the unsupplemented diet with an antibiotic (chlortetracycline) also caused no improvement of growth performance of the broilers. Despite the lack of effect on growth performance, broilers fed the *F. velutipes* stem residue-supplemented diet had a higher antibody titre against Newcastle disease at d 14 of age and higher serum levels of IgG and several cytokines at d 42 of age compared to broilers fed the unsupplemented diet being indicative of an immunostimulatory effect of the *F. velutipes* stem residue. The latter effect has been proposed by the authors to be attributed to the mushroom β-glucans which exert beneficial immunomodulatory effects in the gut through shaping the gut microbiota composition (Araújo-Rodrigues et al. 2024). Based on the observed immunostimulatory effects, feeding *F. velutipes* stem residue might be an effective strategy for improving immune function of broilers. Likewise, no improvement of performance parameters (BW gain, feed conversion) was seen in a study with female Ross 308 broilers, which were fed diets with 5 g/kg diet of stem residues from either *P. eryngii*, *P. sajor-caju*, *F. velutipes* from d 1 to 35 of age (Hsieh et al. 2021). In addition, the counts of selected bacteria, such as *Lactobacillus spp*., *Coliforms* and *Clostridium perfringens*, in ileum and caecum did not differ between groups fed the diets supplemented with mushroom stem residues and the unsupplemented control diet. However, the villus:crypt-ratio in the jejunum was higher in broilers fed the diet with *P. eryngii* stem residues, and the mRNA

levels of cytoprotective genes (e.g. haem oxygenase 1) in peripheral blood mononuclear cells was higher in all broiler groups fed the different mushroom stem residues than in the group of broilers fed the unsupplemented diet. Despite the lack of a growth-promoting effect of the mushroom stem residues, the findings of this study at least suggested that feeding mushroom stem residues is a reasonable and sustainable strategy to improve the capacity of the immune system to respond to oxidative and/or infectious stimuli in broilers.

### 6.2.2. Spent mushroom substrate (SMS)

In contrast to studies with fungal fruiting bodies or fungal mycelia, limited research exists regarding the effect of SMS in broilers. A summary of available studies is shown in Supplementary Table 4. A positive effect on performance (BW gain, feed intake, feed conversion-ratio) was reported for feeding *F. velutipes* SMS at 2 g/kg diet to Ross 308 broilers from d 1 to 35 of age compared to feeding the unsupplemented basal diet (Srinual et al. 2024). At lower doses of the *F. velutipes* SMS (0.5 and 1 g/kg diet), BW gain and feed intake were higher but feed conversion-ratio did not differ from broilers fed the unsupplemented basal diet. Apart from growth performance, caecal microbiota composition was beneficially affected by the SMS considering that broilers fed 0.5, 1 and 2 g/kg diet of SMS had lower caecal counts of *E. coli* and *Salmonella spp*. but higher counts of *Lactobacillus spp*. than broilers fed the unsupplemented diet. Small intestinal morphology, such as the villus height:crypt depth-ratio was not affected by feeding *F. velutipes* SMS. Also, a positive effect was found in a study from Machado et al. (2007), in which broilers fed 2, 4, 6, and 8 g/kg diet of *A. blazei* SMS from d 1 to 42 of age had similar BW gains and feed:gain-ratios as broilers fed a diet with an antibiotic (avilamycin) but higher BW gains and lower feed:gain-ratios than broilers fed the unsupplemented basal diet. Carcass yield and breast yield were highest in broilers fed the *A. blazei* SMS at 2 g/kg diet and broilers fed the diet with the antibiotic. In a recent study the potential of *P. ostreatus* SMS in abrogating the negative effect of replacing soybean meal by combined marula seed cake (MSC) and mucuna seed meal (MSM) in the diet was investigated in Ross 308 broilers (Mthana and Mthiyane 2024). While soybean meal replacement by combined MSC and MSM in the diet reduced feed intake, BW gain, and feed conversion efficiency, slaughter weight, carcass and breast weight, additional supplementation of 12.5 g/kg diet of *P. ostreatus* SMS improved feed intake, BW gain, feed conversion efficiency, slaughter weight, carcass weight and breast weight during the experimental period (d 1–42 of age) compared to broilers fed the replacement diet without SMS. Higher doses of the *P. ostreatus* SMS (25 and 50 g/kg diet) were less efficient than 12.5 g/kg SMS in improving performance and slaughter characteristics of the broilers fed the replacement diet. No effect on performance parameters (BW gain, feed intake, feed conversion-ratio) was observed in a study with male Ross 308 broilers fed different doses (1, 2.5 and 5 g/kg diet) of *C. militaris* SMS compared with broilers fed the unsupplemented basal diet from d 1 to 36 of age (Noopan et al. 2019). Also, feeding diets supplemented with either 5, 10, 15 or 20 g/kg diet of *P. sajor caju* SMS to Ross 308 broilers from d 1 to 38 of age did not alter growth performance when compared to broilers fed the unsupplemented diet (Azevedo et al. 2009). In a further study with broilers, dietary inclusion of *P. ostreatus* SMS at 25, 50, 75 and 100 g/kg diet at the expense of wheat bran caused even a negative effect on growth performance (Foluke et al.

2014). According to this incompletely documented study (breed of the broilers and duration of the feeding period were not mentioned), the feed conversion-ratio increased with increasing inclusion level of SMS, because feed consumption strongly increased while BW gain only slightly increased.

## 7. Conclusions and perspectives

Biomass from cultivated edible mushrooms can be sustainably produced using residual lignocellulosic sidestreams from agri-industrial and wood processing and is a rich source of a variety of bioactive compounds like fermentable carbohydrates, bioactive peptides, vitamins, phenolic compounds and pharmacologically active chemical compounds (e.g. lovastatin), which are likely responsible for the wide array of beneficial metabolic and health-promoting effects of mushroom consumption (Muszyńska et al. 2018). Feeding studies with healthy and parasite-infected broilers using fungal fruiting bodies, mycelia, stem residues or SMS nearly equally showed either positive or neutral effects on growth performance, whereas impairments of performance and carcass parameters were not reported. Improvements of broiler performance and carcass quality in response to feeding fungal biomass were at least partially associated with a beneficial modulation of the gut microbiota community structure, i.e. increased abundance of gut protective and decreased abundance of obligate pathogenic bacterial genera – effects that are most likely attributed to the presence of fermentable polysaccharides (β-glucans, chitin) and phenolic compounds with prebiotic and selective antimicrobial activities, respectively. A large body of evidence exists that such a beneficial modulation of the gut microbiota composition is accompanied by an improved gut barrier integrity, gut function, and systemic health (Ringseis et al. 2020), thereby, enhancing nutrient utilisation, metabolic efficiency and ultimately growth rate. Accordingly, the growth-promoting effect of fungal biomass in broilers might be explained by both, prebiotic and antimicrobial activities as summarised in Figure 1. Based on the evidence from literature presented in this review, the inclusion of fungal biomass, particularly inexpensive by-products from mushroom production like stem residues and SMS, in broiler diets can be recommended, because even if no growth-promoting effects are induced the partial replacement of less sustainably produced feed components by fungal biomass improves the environmental impact of broiler production. However, regarding that the dietary inclusion level of fungal biomass in compound feed for broilers does not exceed 50 g/kg diet, the potential of improving sustainability of broiler production by dietary inclusion of fungal biomass is limited. Considering that the number of broiler studies showing either positive or neutral effects of fungal biomass on growth performance are broadly balanced and divergent study outcomes are reported even for biomass from the same fungal species, future studies are necessary to identify the specific requirements (e.g. content of nutrients and antinutritional compounds in the fungal biomass, type of cultivation substrate, specific combination of fungal species and substrate) of fungal biomass responsible for promoting broiler performance. Despite the above-described studies reported no negative effects of fungal mycelia and fungal extracts on the growth performance of broilers even at the highest dose tested, it has to be kept in mind that indigestible carbohydrates contained in mushrooms are well-known to exert antinutritional effects at higher concentrations, such as decreasing nutrient digestibility due to increasing digesta viscosity (Wang et al. 1992).

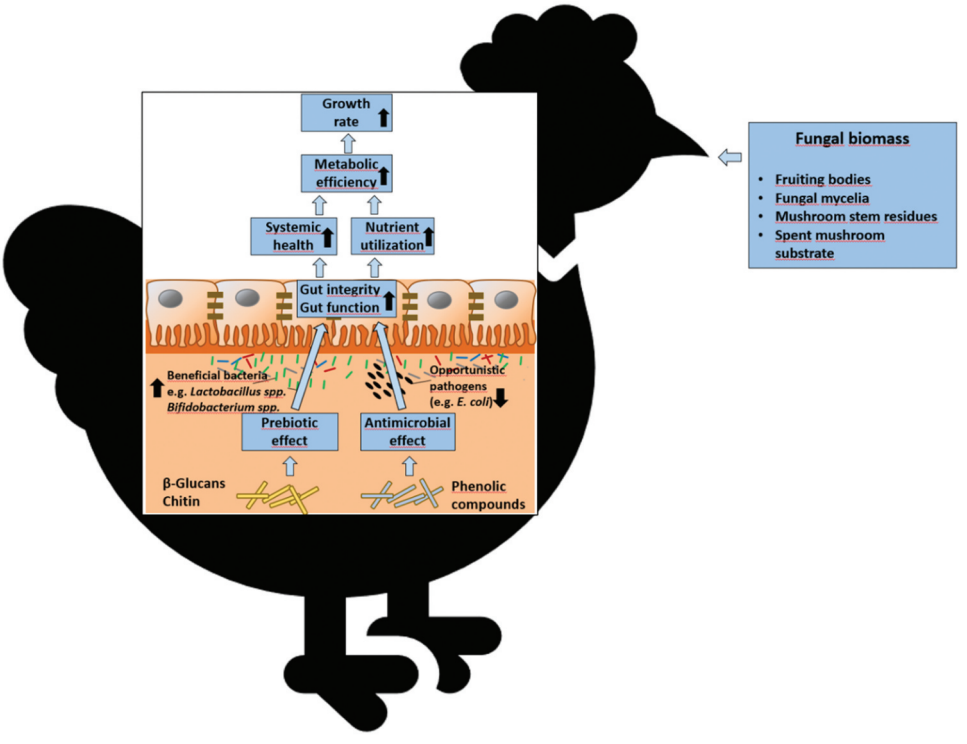


**Figure 1.** Proposed mechanisms underlying the growth-promoting effect of feeding fungal biomass in broilers. Studies with healthy and parasite-infected broilers show that improvements of broiler performance in response to feeding fungal biomass, like fruiting bodies, fungal mycelia, mushroom stem residues and spent mushroom substrate, cause a beneficial modulation of the gut microbiota community structure with increased abundance of gut protective bacteria, such as *Lactobacillus spp*. and *Bifidobacterium spp*., and decreased abundance of obligate pathogenic bacterial genera (e.g. *E. coli*). These effects are most likely mediated by the presence of fermentable polysaccharides, like β-glucans and chitin, and phenolic compounds in fungal biomass, which exhibit prebiotic and selective antimicrobial activities, respectively. A large body of evidence exists that such a beneficial modulation of the gut microbiota composition is accompanied by an improved gut barrier integrity, gut function, and systemic health, thereby, enhancing nutrient utilisation, metabolic efficiency and ultimately growth rate.

Thus, more dose-response studies are necessary in order to closely define the upper inclusion level of fungal biomass which does not impair broiler performance. Finally, future feeding concepts for broilers using fungal biomass must also consider different safety aspects. In this regard it is particularly important that the substrates used for solid-state and submerged fermentation are in accordance with feed law regulations on undesirable substances like heavy metals, mycotoxins and organochlorine compounds. In addition, a potential allergenicity of fungal protein must be taken into account.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

## Funding

The author(s) reported there is no funding associated with the work featured in this article.

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