Solid-State Fermentation of Rice Straw and Agricultural By-products with White-Rot Fungi: A Biological Route to Better Digestibility in Ruminants

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ABSTRACT

Rice and wheat straws form the backbone of dry roughage for Indian dairy animals, yet their lignified cell walls, low protein content and poor digestibility restrict intake and energy supply, and a large share is still burnt in the field. Solid-state fermentation (SSF) with white-rot fungi, chiefly *Pleurotus* species, offers a biological alternative to chemical treatment. Fungal laccase and peroxidases enzymatically loosen lignin, exposing cellulose and hemicellulose to rumen microbes, while fungal biomass raises the crude protein of the straw. Published work reports straw crude protein rising from about 2 percent to between 5 and 6.6 percent, in vitro dry matter digestibility reaching about 65 percent, and better nutrient digestibility in small ruminants, but the response varies widely with fungal strain, moisture, temperature and incubation time. This article reviews the mechanism, a field-

oriented protocol, compositional and animal responses, an illustrative costing for Indian conditions, practical challenges and research priorities. SSF appears most promising where low-cost inoculum such as spent mushroom substrate are used and treated straw is built into balanced rations.

INTRODUCTION:

Feed and fodder account for roughly 70 percent of total production expenses in India and the country is reported to face deficits of about 11 percent in dry fodder, 35 percent in green fodder and 28 percent in concentrates, although estimates differ between agencies (Rath, 2019). These gaps sit beside a national milk output of 247.87 million tonnes in 2024-25 (DAHD, 2025), which means that productive animals are being fed from a thin resource base. At the same time, more than 500 million tonnes of crop residues are generated every year, and a large portion is burnt on the farm to clear the field for the next crop (Rath, 2019). The biomass lost through open-field burning represents a potentially valuable source of dry roughage for the dairy sector, where the availability of quality fibrous feed resources is often limited.

Rice straw contains cellulose and hemicellulose that are potentially fermentable by rumen microorganisms; however, their accessibility is restricted by the lignin matrix, which is resistant to microbial degradation. Its nutritional value is further limited by its low crude protein content, reported as 2.05% in untreated rice straw by Datsomor *et al.* (2022), resulting in reduced intake and digestibility. Urea-ammoniation is a commonly adopted treatment to improve the feeding value of rice straw (Vorlaphim *et al.*, 2021); however, its effectiveness depends on controlled treatment conditions and adequate airtight storage. Solid-state fermentation using white-rot fungi offers a biological approach to improving the nutritive value of rice straw by enzymatically degrading lignin and enhancing fibre accessibility, rather than relying on alkaline treatments to modify the fibre structure. From an applied ruminant nutrition perspective, this article examines the underlying mechanisms, practical implementation under Indian farming conditions, effects on feed quality, economic feasibility, and key limitations of this process.

1. **Principle of SSF and the organisms used:** SSF is the growth of microorganisms on a moist solid material in the absence, or near absence, of free water. For ruminant feeds the purpose is not to make a food product but to modify the substrate so that its structural carbohydrates become available to the rumen. White-rot basidiomycetes are the organisms of choice because they are among the few that can mineralise lignin to carbon dioxide and water (Datsomor *et al.,* 2022). *Pleurotus* species are preferred in practice since they are edible and non-toxic, which removes much of the safety concern attached to mould-based fermentations.

Common applications include rice straw treated with *Pleurotus ostreatus* or *P. sajor-caju* (now classified as *Lentinus sajor-caju*), wheat straw treated with *P. florida*, straw colonised with spent mushroom substrate (SMS), and agro-residues fermented with *Trichoderma* species or mixed microbial consortia. Wang *et al.* (2023) screened six white-rot fungi on highland barley straw: all raised crude protein and lowered NDF, ADF and lignin, yet *L. sajor-caju* performed best. Rothmann *et al.* (2023) likewise reported that fungal species and fermentation duration are critical factors influencing the extent of substrate degradation. They also emphasised that the digestive physiology of the target

animal should be considered when selecting an appropriate bioconversion strategy.

2. **Mechanism of lignin degradation:** Lignin degradation by white-rot fungi occurs primarily through oxidative mechanisms rather than hydrolysis. These fungi secrete extracellular ligninolytic enzymes, including laccase, manganese peroxidase (MnP), and lignin peroxidase (LiP), which generate reactive intermediates that facilitate the breakdown of chemical bonds within the lignin polymer. In several *Pleurotus* species, versatile peroxidase may also contribute to lignin degradation. Modification of the lignin matrix improves the accessibility of cellulose and hemicellulose to rumen microorganisms, potentially enhancing microbial attachment, fibre digestibility, and voluntary feed intake. Furthermore, fungal biomass accumulation may contribute additional protein, thereby increasing the crude protein concentration of the treated straw.

The selectivity of lignin degradation is a critical determinant of treatment effectiveness. Tuyen *et al.* (2012) evaluated eleven fungal species during wheat straw incubation for up to 49 days and reported lignin losses of up to 63%. However, lignin degradation was strongly correlated with hemicellulose loss (r = 0.96). In contrast, improved gas production was strongly associated with the ratio of lignin loss to cellulose loss (r = 0.87). The most effective fungus, *Ceriporiopsis subvermispora*, increased gas production from 200 to 309 mL/g organic matter after 49 days of incubation. These findings highlight the importance of selecting fungal strains that preferentially degrade lignin while minimising the loss of potentially fermentable carbohydrates, thereby maximising the nutritional benefits of biological straw treatment.

3. **A practical protocol for Indian farm conditions:** The protocol below is the non-sterile, commercially feasible version described in the field report supplied for this article and it is summarised in Figure 1. Rice straw is chopped to 2 to 5 cm, and mouldy straw and foreign matter are discarded. It is soaked in clean water for 8 to 12 hours and drained to 60 to 65 percent moisture, then sprayed with a urea solution at 1 kg per 100 kg straw, which supplies nitrogen to the fungus and helps suppress contaminant moulds. *Pleurotus* spawn is mixed in at 2 percent of straw weight (2 kg per 100 kg), and the straw is packed loosely in perforated polythene bags or covered heaps. Aeration is essential because the fungus is strictly aerobic. Incubation at 25 to 30 °C and 70 to 85 percent relative humidity continues until white mycelium covers the straw uniformly, after which the product is sun-dried or ensiled and introduced gradually into the ration. Vorlaphim *et al.* (2021) used closely similar conditions, incubating urea-treated rice stubble with *P. ostreatus* at 25 to 30 °C for 25 days.

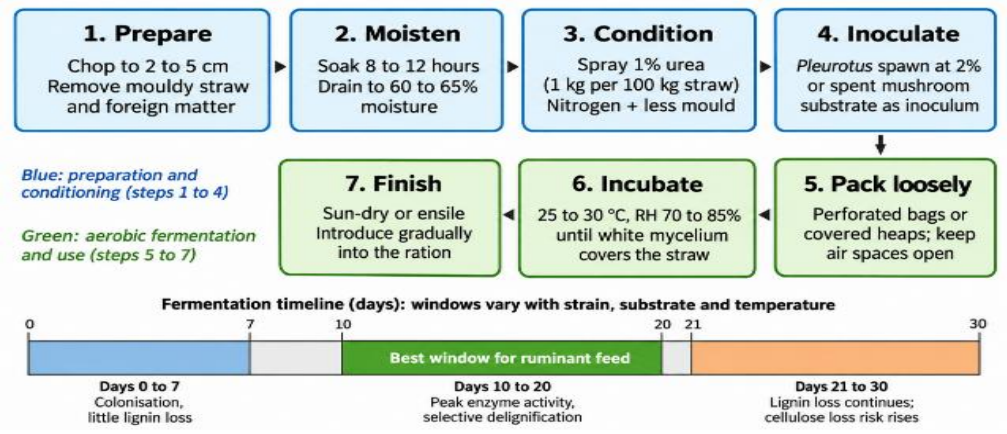


Figure 1. Stepwise SSF protocol for rice straw with the three phases of fermentation

The fermentation process can be broadly divided into three phases. During the first 7 days, fungal mycelium progressively colonises the straw and initiates ligninolytic enzyme production, although substantial lignin degradation may not yet occur. Between days 10 and 20, enzymatic activity may reach its peak, facilitating selective delignification while largely preserving cellulose, thereby representing a potentially favourable period for producing ruminant feed. Beyond day 21, continued lignin degradation may be accompanied by increasing cellulose losses, potentially reducing the nutritional value of the treated substrate. Although the field protocol specifies a working incubation period of 25 to 30 days, it identifies days 10 to 20 as the optimal window. Therefore, producers should begin evaluating the substrate at approximately day 14 and determine the termination point based on fungal colonisation and substrate quality, rather than relying exclusively on a fixed duration, while avoiding incubation beyond 30 days without supporting evidence. Prolonged fermentation should be approached cautiously because Martens *et al.* (2023), in a 42-day study involving wheat straw under mushroom cultivation conditions, reported declining in vitro indices of ruminal degradability over time with all three fungal species evaluated. Furthermore, nitrogen supplementation and incubation temperature require optimisation to maximise delignification while minimising carbohydrate losses. Wang *et al.* (2023) reported a maximum lignin reduction of 50.19% under optimised conditions involving 2.29% urea, 1.53% glucose, and an incubation temperature of 22.72 °C.

4. Effect on fibre fractions and digestibility

Figure 2 summarises the indicative ranges given in the field report. NDF falls from 70 to 75 percent to 60 to 65 percent, ADF from 45 to 50 percent to 38 to 42 percent, and acid detergent lignin is reduced by 15 to 30 percent, while crude protein rises from 2 to 3 percent to 5 to 7 percent and in vitro dry matter digestibility (IVDMD) from 40 to 45 percent to 50 to 65 percent. In rice straw, Datsomor *et al.* (2022) found crude protein rising from 2.05 percent to 5.24 percent with *P. ostreatus* alone and to 6.58 percent when the straw was ammoniated before fungal treatment. The combined treatment also gave the highest IVDMD (65.52 percent against 56.16 percent for ammoniation alone), total volatile fatty acids (76.56 against 67.71 mM) and gas production (56.78 against 44.30 ml per g), which suggests that urea and fungus act in a complementary way.

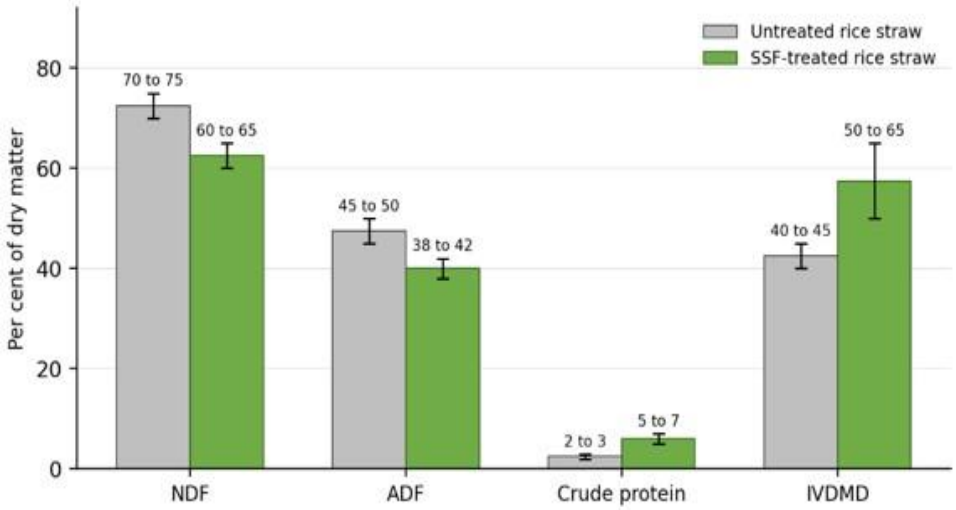


Figure 2. Indicative NDF (neutral detergent fibre), ADF (acid detergent fibre), crude protein composition and in vitro dry matter digestibility (IVDMD) of untreated and SSF-treated rice straw.

Two important limitations should be considered when interpreting the nutritional improvements associated with fungal treatment. First, the apparent increase in crude protein may partly reflect a concentration effect resulting from carbohydrate loss through fungal respiration and the release of carbon dioxide, rather than an equivalent increase in the absolute protein content of the substrate, as suggested by the relative increases reported by Wang *et al.* (2023). Second, treatment outcomes vary considerably among studies, depending on fungal species, substrate characteristics, incubation conditions, and fermentation duration, as summarised in Table1.

Table 1. Evidence from selected studies on fungal treatment of straw and agricultural by-products

Study	Substrate and organism	Conditions	Main findings
Datsomor *et al.* (2022)	Rice straw; *P. ostreatus* with or without ammoniation	SSF; in vitro rumen fermentation	CP 2.05% (control), 5.24% (fungus), 6.58% (ammoniation + fungus); IVDMD 65.52% in combined treatment
Wang *et al.* (2023)	Highland barley straw; six white-rot fungi	SSF; in vitro digestibility	All fungi raised CP and lowered NDF, ADF, ADL; *L. sajor-caju* best; lignin loss up to 50.19% under optimised urea, glucose and temperature
Vorlaphim *et al.* (2021)	Rice stubble, 35% of TMR; *P. ostreatus* + urea; goats	25 days at 25 to 30 °C; 12-week feeding trial	Versus untreated stubble: digestibility of OM +8.5%, CP +5.5%, NDF +39.2%, ADF +27.4%; lower feed conversion ratio
Tuyen *et al.* (2012)	Wheat straw; eleven white-rot fungi	24 °C, 0 to 49 days	Lignin loss up to 63% but closely tied to hemicellulose loss; selectivity ratio predicted gas production
Martens *et al.* (2023)	Wheat straw; three fungi under mushroom-farm conditions	Up to 42 days	In vitro NDF digestibility, soluble organic matter and gas production declined with time, by up to 30, 35 and 50%

5. Animal performance

The strongest in vivo evidence comes from small ruminants. In the goat trial of Vorlaphim *et al.* (2021), eighteen animals received total mixed rations containing 35 percent rice stubble that was untreated, urea-treated or treated with urea and *P. ostreatus*; the fungal treatment improved digestibility and gave the lowest feed conversion ratio. Mustabi *et al.* (2013) likewise evaluated white-rot fermented rice straw as an alternative to elephant grass in goat feeds. For dairy cattle the field report indicates 10 to 20 percent higher voluntary intake, improved rumen fermentation, a partial saving of about 0.5 kg concentrate per cow per day and better feed efficiency in low-input systems. Lactation trials with Indian crossbred cows and buffaloes are scarce, and they should be treated as hypotheses until such trials are done. Treated straw should enter the ration gradually, replace only part of the untreated roughage, and be inspected for mould and off-smell before feeding.

6. Field-level challenges

Most on-farm failures of solid-state fermentation (SSF) arise from inadequate process control rather than inherent limitations of this concept. Table 2 summarises the major operational challenges, their potential consequences, and recommended corrective measures. Temperature management is

particularly important under Indian climatic conditions. Hai *et al.* (2023) reported that three white-rot fungal species exhibited growth on rice straw at temperatures up to 35 °C, whereas no growth occurred at 40 °C. Therefore, elevated ambient temperatures and excessive heat accumulation within fermentation heaps during summer may inhibit fungal growth or cause culture failure, highlighting the need for appropriate temperature monitoring and control.

Table 2. Common problems in on-farm SSF, their effects and remedies

Problem	Consequence	Remedy
Costly or poor-quality spawn (Rs 80 to 150/kg; old, contaminated, low viability)	Higher cost; patchy colonisation	Buy from reliable laboratories, check date and viability; use SMS (spent mushroom substrate) as inoculum
Moisture above 70%	Anaerobic pockets, foul smell, spoilage	Drain to 60 to 65% before inoculation
Moisture below 55%	Poor fungal growth; slow lignin degradation	Re-wet evenly; hold humidity at 70 to 85%
Unhygienic site	Contamination by unwanted fungi and moulds	Clean, shaded site; urea spray; discard mouldy straw
Heat above 40 °C inside heap	Mycelium killed	Thin heaps, shade and ventilation; run batches in cooler months
Over-compaction	Oxygen limitation for a strictly aerobic fungus	Pack loosely in perforated bags
Incubation beyond 30 days	Loss of cellulose and hemicellulose	Sample from day 14; stop by day 30
Space and infrastructure	Need for a shaded area protected from sun, rain and animals	Shared units run by cooperatives or producer groups
Scale and cost	Economical mainly at large scale or with low-cost inoculum	Clustered production; SMS from mushroom farms

7. Future perspectives and directions

Development of low-cost inoculum sources using locally available materials is essential. Spent mushroom substrate (SMS) from the expanding mushroom industry could reduce inoculum-related costs; however, its fungal viability, colonisation efficiency, shelf life, and microbial contamination require standardisation. Second, screening and selecting fungal strains adapted to Indian agro-climatic conditions should be prioritised, with particular emphasis on heat tolerance and selective lignin degradation. As demonstrated by Tuyen *et al.* (2012), the ratio of lignin loss to cellulose loss is a useful indicator of potential nutritional improvement.

Replicated trials involving crossbred dairy cattle and buffaloes should evaluate feed intake, milk yield and composition, rumen fermentation characteristics, methane emissions, and economic feasibility. Life-cycle assessment should also compare fungal treatment with conventional residue disposal practices, including open-field burning.

CONCLUSIONS

Solid-state fermentation using *Pleurotus* and other white-rot fungi offers a promising approach to improving straw digestibility through selective lignin degradation and fungal biomass enrichment. However, its effectiveness depends on fungal strain, moisture, temperature, and incubation duration, while validation under dairy cattle feeding conditions is necessary. For practical adoption in India, decentralised treatment systems using low-cost inoculum, including spent mushroom substrate (SMS), could improve economic feasibility and enable the incorporation of treated straw into balanced ration.

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