ORIGINAL ARTICLE

Bioconversion of grape pomace waste into suitable alternative feed for ruminants with *Pleurotus cornucopiae* and *Ganoderma resinaceum* via solid-state fermentation bioprocess

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Abstract

Grape pomace is a polluted waste that is usually deposited in landfills, disrupting plant growth and polluting water. The present study aimed to convert this abundant biomass into a suitable feed for ruminants using white-rot fungi via a solid-state fermentation bioprocess. For this purpose, two white-rot fungi, *Ganoderma resinaceum* (medicinal fungus) and *Pleurotus cornucopiae* (edible fungus), and different durations of solid-state fermentation (0, 4, and 8 weeks) were used to determine the best fungus and the optimal duration of solid-state fermentation. The results showed that fermentation of grape pomace with both white-rot fungi reduced lignin and condensed tannin content and increased crude protein and mineral content. This bioprocess improved rumen fermentation and dry matter and fiber digestibility. This enhanced concentration of volatile fatty acids and ammonia-nitrogen in the rumen which contribute to better microbial crude protein synthesis and metabolizable energy by ruminants. Fermentation of these wastes with *P. cornucopiae* better reduced lignin content and improved rumen fermentation and metabolizable energy than with *G. resinaceum*. However, increasing the fermentation periods with both white-rot fungi reduced the gain of metabolizable energy and ruminal microbial crude protein synthesis. In conclusion, fermentation of grape pomace waste inoculated with *P. cornucopiae* after 4 weeks is the better strategy to bio-convert into suitable ruminant feed. Reduction of feed costs, overcoming of disposal problems of this waste, and provision of a new source of income for the grape juice and wine industry can be achieved through this alternative feed.

Keywords Grape pomace waste · *Ganedorma resinaceum* · *Pleurotus cornucopiae* · Ruminant feed · Bioconversion

1 Introduction

Grapes are one of the most important fruit crops in the world, with over 74.8 million tons, of which about 52% were pressed for wine and juice production in 2021 [1]. This agro-industrial process generates a large amount of waste, composed of 72% of the skins, 17% of the seeds, and 11%

of the stems. Collectively, these components are referred to as grape pomace [2]. This waste represents about 27% of the pressed grapes and is estimated at 7 million tons per year worldwide [2, 3]. Only a small fraction of this waste is recycled or dried for compost production and used as fertilizer or used as traditional fuel, resulting in the release of hazardous pollutants [4]. The burning and dumping of these wastes leads to the impairment of plant growth and water contamination [3]. Finding an environmentally friendly strategy for managing these large quantities of polluted wastes poses several challenges to the environment. Currently, these wastes receive special attention due to their nutraceutical, medicinal, and health benefits, as they contain abundant and inexpensive polyphenols, especially high amounts of flavonoids and anti-oxidant pectin [5–7] Polyphenols are used in the treatment of diabetes, cancer, and cardiovascular diseases because of their high antioxidant and anti-inflammatory properties [8].

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The incorporation of grape pomace at 10% of the total sheep feed improves the nutraceutical quality, fatty acid composition, oxidative stability of lamb meat [9], the nutraceutical quality of ewes milk [10], and ram sperm quality, and mitigates oxidative stress [11]. However, using grape pomace as an alternative feed for sheep has several limitations and potential hazards. The major limitation of using these wastes as an alternative feed for sheep is their very low digestibility, as only 32% of their organic matter, 30% of their crude protein, and 15% of their natural detergent fibers are digestible [12] due to their high content of lignin and condensed tannin [13]. Other limitations associated with the use of these wastes in sheep feeding are occasionally noted, such as the chemical hazards due to residues of pesticides used in viticulture and biological hazards such as the presence of mycotoxins resulting from improper waste storage [7]. Moreover, only 3% of this waste is recycled as animal feed worldwide [14].

Various physical and chemical processing methods such as hydrothermal treatment, ammonia, acids, and alkaline media have been proposed to break down the lignin structure and release holocellulose, making it accessible to rumen microorganisms to improve rumen degradability and nutritional value of lignocellulosic biomass such as cereal straw. Despite the advantages of chemical methods, their negative environmental impact, cost, and required safety technology limit their application [15]. Recently, biological research has proven that white-rot fungi can efficiently degrade lignin in lignocellulosic biomass to CO_2 and H_2O because they are able to produce extracellular lignin-modifying enzymes such as laccases, lignin peroxidase, manganese peroxidase, and versatile peroxidases [16]. Solid-state fermentation is considered a cost-effective and environmentally friendly system that utilizes the capacity of these white-rot fungi for delignification lignocellulosic biomass with low water consumption where the substrate itself is required to contain the necessary moisture to allow the microorganism to survive and grow which consequently lowers bacterial contamination [8, 17]. Compared to submerged fermentation, solid-state fermentation is a smaller fermentation system and provides better yield and product quality, which is gradually preferred [8]

This biological treatment is attracting the attention of animal nutrition researchers who have demonstrated the potential of the genus *Ganoderma* (medicinal white-rot fungus) such as *G. lucidum* to improve rumen fermentation and digestibility of oil palm empty fruit stalks and oil palm fronds [18, 19] and genus *Pleurotus* (edible white-rot fungus) such as *P. ostratus*, *P. eryngii*, and *P. florida* to improve rumen digestibility of lignocellulosic biomass through solid-state fermentation [20]. Moreover, these fungal species were found to significantly increase the polyphenol content of fermented biomass [21, 22], leading an improvement in antioxidant properties [23]. These results highlight the potential of white-rot fungi as valuable tools to improve biomass utilization and enhance their nutritional and antioxidant properties in ruminant diets. In vivo studies demonstrated the ability of this bioprocess to improve growth rate and feed conversion of goats fed rice straw fermented with *P. ostreatus* [24] and to safely increase milk production of dairy goats fed rice straw fermented with *P. sajor-caju* [25].

However the ability of most species of the genus *Pleurotus* and *Ganoderma* to convert lignocellulosic biomass into rumen feed has not been studied, e.g., *P. cornucopiae* and *G. resinaceum*. The study of *P. cornucopiae* and *G. resinaceum* as fungi for the transformation of lignocellulosic waste by solid-state fermentation represents a very innovative and unexplored approach in the field of scientific research and a research avenue that can be new explored. Although there are many studies demonstrating the ability of white-rot fungi to convert various lignocellulosic biomass into suitable alternative feed for ruminant, there is little information on the effectiveness of this strategy on the nutritional value of grape pomace. Recently, Mhlongo et al. [14] demonstrated that fermentation of grape pomace waste with high dose of *P. ostreatus* spawn by solid-state fermentation for 4 weeks degraded their lignin compounds and improved their effective rumen degradability. Indeed, this strategy has environmental implications and extends to economic and industrial dimensions, as it promotes a circular economy model in which waste is converted into valuable resources, represent a paradigm shift in waste management strategies. This regenerative approach is intended to contribute to a more sustainable future that balances economic prosperity and ecological sustainability. However, the effectiveness of this bioprocess depends on fungal species, biochemical properties of the substrate, fermentation duration and culture conditions [19, 20, 26].

Therefore, we hypothesized that solid-state fermentation of grape pomace with *P. cornucopiae* and *G. resinaceum* may improve their nutritional value. With this in mind, the objective of this study was to investigate the effective white-rot fungi and optimal fermentation duration to achieve better bioconversion of this waste to alternative feeds for ruminants.

2 Materials and methods

2.1 Preparation of the white-rot fungi

Two white-rot fungi used in this study, *P. cornucopiae* (accession number: MK422157) and *G. resinaceum* (accession number: MK422153), were provided by collections of basidiomycete white-rot fungi at the Higher Institute of

Applied Biological Sciences of Tunis. These fungi were inoculated into Petri dishes containing 25 ml of an autoclaved, sterilized malt agar medium consisting of 10 g of malt extract and 10 g of bacteriological agar/l, with a 1 cm² piece of the fungi, and incubated for 7 days at 25 ± 1 °C, with mycelia colonizing the entire malt extract agar surface, and stored at 4 °C until incubation of grape pomace.

2.2 Collect of grape pomace and solid-state fermentation with white-rot fungi

Samples of red grape pomace were harvested from by La Cave de Thibar (Thibar, Beja, Tunisia) brought to the laboratory. These agro-industrial wastes were dried at 50 °C until constant weight. Eighteen bottles (experimental units), each containing 200 g of dried grape pomace, were 60% moistened with distilled water and sterilized in an autoclave at 121 °C for 20 min under a pressure of 2 bar. After cooling to 25 °C, 9 autoclave bottles containing grape pomace were incubated with 4 small pieces (1 cm²) of 7-day-old culture mycelium of *P. cornucopiae* on malt extract agar, other 9 autoclave bottles containing grape pomace were also incubated with 4 small pieces (1 cm²) of 7-day-old culture mycelium of *G. resinaceum* on malt extract agar. These bottles were incubated aerobically at a temperature of 25 ± 1 °C under static conditions and excluding light. Samples of 3 bottles for each treatment were taken at 0 (as a control), 4, and 8 weeks after inoculation, immediately oven dried at 50 °C with constant weight to stop fungal growth, and then ground to 1 mm using a Wiley hammer mill. The ground samples were then stored in glass vials with tight-fitting caps to prevent exposure to moisture and air. Storage was in a cool, dry and dark environment at room temperature of 25 °C. These measures were taken to ensure the stability of the samples for further analysis.

2.3 Chemical composition

Samples treated grape pomace with different fungi at different periods were analysed for crude protein (CP, N × 6.25), ether extract (EE), and ash according to the standard method proposed by the Association of Official Chemists Analytical Chemists [27]. Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined using the ANKOM²²⁰ fibre analyzer (ANKOM technology, Macedon, NY, USA) according to the method described by Van Soest et al. [28]. Condensed tannin (CT) contents were determined according to the method described by Makkar [29]. Hemicellulose (HC) content was calculated as the difference between NDF and ADF, and cellulose (CE) content was calculated as the difference between ADF and ADL. Non-fibrous carbohydrate (NFC) content was calculated according to Equation 1

$$NFC = 100 - (NDF + CP + EE + Ash) \qquad (1)$$

2.4 In vitro ruminal incubation

In vitro rumen fermentation of grape pomace was determined by the reading pressure technique according to the protocol of Theodorou et al. [30]. Briefly, rumen inoculum was collected from 4 freshly slaughtered male Noire de Thibar lambs (~9 months old and 32 kg body weight). The lambs were fed a diet consisting of 1 kg of oat hay (with a composition of 74 g of crude protein, 538 g of neutral detergent fiber, 380 g of acid detergent fiber, 82 g of acid detergent lignin, and 85 g of ash per kg dry matter) and 1 kg of commercial concentrate (with a composition of 143 g of crude protein, 187 g of neutral detergent fiber, 94 g of acid detergent fiber, 13 g of acid detergent lignin, and 64 g of ash per kg dry matter). Rumen inoculum was collected from different sites within the rumen of these lambs. The rumen inoculum was filtered through four layers of cheesecloth and rapidly transported to the laboratory in thermos flasks previously filled with distilled water at 39 °C to avoid temperature shock to the rumen fluid. In addition, the rumen inoculum was flushed with CO_2 to ensure that the environment remained anaerobic. In the laboratory, artificial buffer solution with pH of 6.8 was prepared as described by Menke and Steingass [31]. This artificial buffer solution was constated by 237 ml of macro-mineral solutions ($Na_2HPO_4.12H_2O$, 14.37g; KH_2PO_4, 6.2g and $MgSO_4.7H_2O$, 0.6g dissolved in 1 liter of distilled water), 0.12 ml of micro-mineral solutions ($CaCl_2.2H_2O$, 13.2g; $MnC_{12}.4H_2O$, 10g; $CoC_{12}.6H_2O$, 1g; $FeC_{13}.6H_2O$, 8g dissolved in 0.1 liter of distilled water), 237 ml of buffer solution (($NaHCO_3$, 35g; NH_4HCO_3, 4g dissolved in 1 liter of distilled water), 1.22 ml potential redox indicator ($C_{12}H_6NO_4$ (resazurine), 0.1g dissolved in 0.1 liter of distilled water), 475 ml of distilled water and reducing agent ($Na_2S.5H_2O$, 0.235g; NaOH (1N), 2ml; dissolved in 0.0475 liter of distilled water) add until discolored. The rumen inoculum was mixed with an artificial buffer solution (1:2 ratio) prepared according to the method of Menke and Steingass [31] at 39 °C, continuing to be flushed with CO_2 at 39 °C. Samples of 200 milligrams of dry grape pomace with appropriate treatment were incubated with 30 mL of the buffered rumen inoculum in prewarmed (39 °C) sterile amber glass serum bottles of 120 mL. Amber glass serum bottles containing only 30 mL of the buffered rumen inoculum were used as negative controls to correct for gas production from the buffered rumen inoculum. All flasks were immediately sealed with a rubber cap and an aluminum crimp cap and incubated in a shaking water bath at a constant temperature of 39 °C. All incubations were performed in triplicate. The gas pressure in the headspace of each fermentation bottle was measured at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours after incubation by inserting a 23-gage needle attached to a pressure transducer (model

PX4200-015GI, Omega Engineering, Inc., Laval, QC, Canada) into the rubber stoppers of the serum bottles. The needles were left on the serum bottles after insertion to allow all available gas to escape from the glass serum bottle.

Gas pressure readings were converted to gas volume using Equation 2 described by Mauricio et al. [32]:

$$\text{Gas volume (mL)} = \left[\text{Gas pressure (psi)} \times 4.8843\right] + 3.1296 \tag{2}$$

The data of cumulative volume of biogas production generated were fitted with an exponential mathematical model according to Equation 3 determined by France et al. [33] using the nonlinear package from SAS 9.3 version; SAS Institute Inc [34]:

$$GP_{(t)} = B\left(1 - e^{-C\,(t-\text{Lag})}\right) \tag{3}$$

Where GP is net gas production (mL/g dry matter); t is incubation time (hours); B is asymptotic gas production (mL/ g dry matter); C is constant gas production rate (mL/ hours); and Lag is time of onset of rumen fermentation (hours).

At the end of fermentation, rumen pH was immediately measured using a pH meter (Jenway Ltd Felsted, model 3020, England). The contents of each serum bottle were filtered using filter paper disks (Whatman 541). The residues were collected and their dry matter and NDF were determined. In vitro dry matter digestibility and in vitro NDF digestibility were determined according to Equation 4 and Equation 5, respectively:

$$\text{In vitro dry matter digestibility (\%)} = \frac{\text{initial dry matter} - \text{residual dry matter}}{\text{initial dry matter}} \times 100 \tag{4}$$

$$\text{In vitro NDF digestibility (\%)} = \frac{\text{initial NDF} - \text{residual NDF}}{\text{initial NDF}} \times 100 \tag{5}$$

Samples of 4 mL of supernatant fluid from each bottle were preserved by adding 2 mL of 1 N H_2SO_4 to determine rumen ammonia-nitrogen by the phenol-hypochlorite method at 630 nm absorbance, as described by Broderick and Kang [35].

Metabolizable energy, short-chain fatty acids, and microbial crude protein were estimated from gasses formed after 24 h of incubation according to the equations 6, 7, and 8 proposed by Menke and Steingass [31], Getachew et al. [36], and Blümmel et al. [37], respectively:

$$\text{Metabolizable energy} = 2.2 + 0.136 \times GP24 + 0.057 \times CP \tag{6}$$

$$\text{Short} - \text{chain fatty acids} = -0.00425 + 0.0222 \times GP24 \tag{7}$$

$$\text{Microbial crude protein} = \text{amount of digestible dry matter} - 2.2 \times GP24 \tag{8}$$

Metabolizable energy in MJ per kg dry matter; short-chain fatty acids in mmol/200 mg dry matter; microbial crude protein in mg/ g dry matter; GP24 is net gas production after 24 hours of incubation in mL/ 200 mg dry matter; CP is crude protein in percent of dry matter and amount of digestible dry matter in mg/ g at the end of incubation.

2.5 Statistical analysis

All collected data were statistically analyzed using the general linear model of SAS 9.3 version; SAS Institute Inc [34], flowing the static model:

$$Y_{ijk} = \mu + \text{periods}_i + \text{fungi}_j + (\text{periods} \times \text{fungi})_{ij} + \varepsilon_{ijk}$$

Where: μ is the overall mean, periods $_i$ is period of incubation in week (i =0, 4 and 8) , fungi $_j$ is white-rot fungi (*P. cornucopiae* and *G. resinaceum*), periods $\times$ fungi $_{ij}$ is the interaction between periods $_i$ and fungi $_j$ and ε_{ijk} is the residual experimental error. The difference between the means of the treatments was compared using Tukey's multiple range test. In all statistical tests, the differences were considered significant when the *P*-value <0.05. All data for each treatment and parameter were obtained from nine replicates (*n* = 9) in this study.

3 Results and dissection

3.1 Chemical composition

The chemical composition of the grape pomace waste used in this study is shown in Table 1. It is comparable to the chemical composition of grape pomace found in previous studies in different geographical areas [11–14, 38]. These abundant agro-industrial wastes are characterized by high content of natural and acid detergent fibers. These fibers are of great interest in ruminant diets because they maintain rumen homeostasis by promoting proper rumen fermentation and supporting overall ruminant health and performance. Prolonged chewing of the fibers stimulates saliva production, which is rich in bicarbonates and phosphates that act as natural buffers to maintain rumen pH, prevent the risk of rumen acidosis and support optimal rumen fermentation. Fiber serves as a valuable substrate for enhancing the cellulosic microbiota in the rumen, which promotes the production of cellulolytic enzymes, leading to more efficient breakdown of fiber into simpler compounds, including volatile fatty acids. In addition, the high fiber feed prolongs rumination time and promotes

Table 1 Chemical composition of grape pomace treated with white-rot fungi

	Week	CP	NDF	ADF	ADL	EE	ASH	NFC	CE	HC	CT
Pleurtus cornucopiae	0	10.3[b]	58.7[a]	45.3[a]	26.5[a]	9.5	9.3[c]	12.2[c]	18.9[a]	13.3[a]	1.5[a]
	4	12.4[a]	50.6[bc]	37.7[b]	18.9[c]	9.5	10.5[c]	17.0[a]	18.8[a]	12.9[a]	1.1[b]
	8	12.8[a]	49.9[bc]	36.8[bc]	18.3[c]	9.7	14.8[b]	12.8[c]	18.5[a]	13.1[a]	1.0[b]
Ganoderma resinaceum	0	10.3[b]	58.9[a]	45.5[a]	26.4[a]	9.4	9.3[c]	12.1[c]	19.1[a]	13.4[a]	1.5[a]
	4	12.3[a]	52.7[b]	39.3[b]	20.7[b]	9.5	9.9[c]	15.6[ab]	18.6[a]	13.4[a]	1.2[b]
	8	12.9[a]	47.2[c]	35.3[c]	20.2[b]	9.6	16.2[a]	14.1[b]	15.1[b]	11.9[b]	1.0[b]
SEM		0.33	1.21	1.01	0.98	0.22	0.66	0.88	0.99	1.01	0.03
P-value	Periods	***	***	***	***	NS	***	***	*	**	**
	Fungi	NS	NS	NS	**	NS	NS	*	*	**	NS
	Periods × fungi	NS	NS	NS	*	NS	NS	**	**	**	NS

[a,b,c]Means value flowed by different superscript in same column differed at $P < 0.05$ (Tukey test); *SEM*, standard error of means; ***P-value <0.001; **P-value < 0.01; *P-value < 0.05; NS; P-value >0.05; *CP* crude protein (% dry matter), *NDF* neutral detergent fiber (% dry matter), *ADF* acid detergent fiber (% dry matter), *ADL* acid detergent lignin (% dry matter), *EE* ether extract (% dry matter; ash (% dry matter), *NFC* non-fiber carbohydrate (% dry matter)), *CE* cellulose (% dry matter), *HC* hemicellulose (% dry matter), *CT* condensed tannin (% dry matter)

better digestion and fermentation[15]. The crude protein contained in the wastes can ensure the normal function and growth of the rumen microbiota, which is responsible for the degradation of complex plant materials such as fiber, and promotes efficient rumen fermentation leading to optimal production of volatile fatty acids and microbial protein. When rumen microorganisms metabolize nitrogen provided by crude protein, they synthesize essential amino acids that are then absorbed and utilized by the ruminant. These amino acids are the basic building blocks of proteins and serve as vital components for tissue development and growth, muscle and organ formation, and tissue repair and maintenance. They are used to form enzymes that enable biochemical reactions in the body that are critical for digestion, metabolism and nutrient utilization. It is also used to form hormones and other signaling molecules that regulate various physiological processes and maintain homeostasis. In this way, adequate crude protein content in feed optimizes ruminant performance and productivity [39]. Based to the fiber and crude protein compounds, these wastes can be used as roughage for small ruminants better than cereal straw [40]. However, their high content of lignin compounds form a physical obstruction of access of the rumen microbiota and its digestive enzymes to degrade other compounds [15], and their high content of condensed tannin reduces the proliferation of rumen microbiota and inhibits its enzyme activity, which reduces their nutritional value [41, 42].

Fermentation of grape pomace waste with *P. cornucopiae* reduce their lignin compounds by 29% and 31% after 4 and 8 weeks, respectively. This delignification is better than that found by Mhlongo et al. [14], on the grape pomace fermented with *P. ostreatus* spawn after 4 weeks. Therefore, it is more effective to treated grape pomace with *P. cornucopiae* than *P. ostreatus*. Since the effectiveness of this bioprocess is related to the species of *Pleurotus* [20]. Fermented grape pomace with *G. resinaceum* also reduced their lignin compounds by 21% and 23% after 4 and 8 weeks, respectively. To our knowledge, this is the best result found in delignification of highly lignified biomass with the genus *Ganoderma*. Only at longer periods (12 weeks) Van Kuijk et al. [43] show a higher yield of delignification of less lignified biomass such as wheat straw fermented with *G. lucidum*. The better capacity of *P. cornucopiae* to degrade lignin compounds than with *G. resinaceum* is in agreement with the findings of Van Kuijk et al. [43], who demonstrated that species belonging to *Ganoderma* genus have a lower ability to degrade the lignin complex of wheat straw and Miscanthus than species of the genus *Pleurotus*. Prolongation of this bioprocess with both fungi did not allow further reduction of lignin content of grape pomace. A similar effect was noted by Nur-Nazratu et al. [19] in empty fruit bunch inoculated with *G. lucidum* at 4 to 8 weeks. This result may be attributed to the high increase in crude protein compound in the substrate and the capacity of production of lignin enzymes in serval white-rot fungi is produced under nitrogen limitation conditions [44]. Only *G. resinaceum* reduced the cellulose and hemicellulose compounds of this waste after 8 weeks of fermentation. Nur-Nazratu et al. [19] also mentioned that the fermented palm empty fruit bunch with *G. lucidum* significantly dissolved their hemicellulose compounds after 6 weeks and cellulose compounds after 10 weeks. This result can be explained by the increased availability of nutrients, namely in crude protein, in the colonized substrate, which caused a shift in the synthesis of ligninolytic enzymes to cellulolytic enzymes [18]. The ability of *P. cornucopiae* to degrade lignin during this bioprocess without altering the cellulose fraction is very interesting for ruminant nutrition, since the ruminant microbiota is able to convert cellulose and hemicellulose into volatile fatty acids, which are the

Table 2 Rumen fermentation characteristics of grape pomace treated with white-rot fungi

	Week	B	C	Lag	DMD	NDFD	pH	NH$_3$-N	ME	VFA	MCP
Pleurtus cornucopiae	0	138.9[c]	0.036[c]	2.11[a]	37.7[c]	20.1[c]	6.47[a]	20.54[c]	4.84[c]	0.332[d]	344[c]
	4	175.3[a]	0.044[a]	0.95[b]	44.0[a]	26.3[a]	6.37[b]	24.71[b]	5.95[a]	0.492[a]	391[a]
	8	162.4[b]	0.040[b]	1.02[b]	42.2[ab]	25.2[a]	6.44[a]	26.6 [a]	5.58[b]	0.429[b]	379[b]
Ganoderma resinaceum	0	139.1[c]	0.035[c]	2.09[a]	37.7[c]	20.2[c]	6.47[a]	20.52[c]	4.83[c]	0.329[d]	344[c]
	4	161.3[b]	0.041[ab]	0.99[b]	43.3[a]	24.2[ab]	6.46[a]	24.66[b]	5.58[b]	0.433[b]	397[a]
	8	156.4[b]	0.038[bc]	1.01[b]	40.1[b]	23.4[b]	6.46[a]	25.95[a]	5.41[b]	0.400[c]	372[b]
SEM		2.04	0.002	0.451	0.55	0.42	0.020	3.34	0.12	0.011	8.1
P-value	Periods	***	***	***	***	***	*	***	***	***	***
	Fungi	*	*	NS	NS	*	*	NS	**	**	NS
	Periods × fungi	*	NS	NS	*	NS	**	NS	NS	NS	NS

[ab,c]Means value flowed by different superscript in same column differed at $P < 0.05$ (Tukey test); *SEM*, standard error of means; ***P-value <0.001; **P-value < 0.01;*P-value < 0.05; NS; P-value >0.05; *B*, amount of rumen fermentation (mL/g dry matter); *C*, rate of rumen fermentation (mL/h); *Lag*, time of the onset of rumen fermentation (h); *DMD* dry matter digestibility (%), *NDFD* neutral detergent fiber digestibility (%), *NH$_3$-N* rumen ammonia-nitrogen (mg/L), *ME* metabolizable energy (MJ/kg dry matter), *VFA* volatile fatty acids (mmol/ 200 mg dry matter), *MCP* microbial crude protein (mg/ g dry matter)

main source of their growth and proliferation and thus promoting ruminant performance [45, 46]. This bioprocess increased crude protein compound of grape pomace. Consistent with our findings, Mhlongo et al. [14] found a net increase in crude protein of grape pomace fermented with various doses of *P. ostreatus* spawn. This gain could be due to the hydrolysis of carbohydrates to CO_2 and H_2O, resulting in a loss of organic matter but not crude protein [47], the synthesis of protein-rich fungal mycelial biomass during fermentation [40], the ability of fungi to synthesize proteins from nitrates and amines in fermentation substrates [48], the increase in chitin, a component of fungal cell walls composed of N-acetyl and β-glucan, which also contains N [49], the higher uptake of atmospheric nitrogen during mycelial growth [20]. The effects of white-rot fungi on the condensed tannins have rarely been investigated. To our knowledge, this is the first study to investigate the effect of the genus *Ganoderma* on tannin compounds. This study has proved the capacity of both white-rot fungi to reduce this antinutritional compound after 4 weeks by 27% and 20% with *P. cornucopiae* and *G. resinaceum*, respectively and 33% after 8 weeks for both white-rot fungi. This result is in accordance with previous studies which proved the capacity of *P. ostreatus* to decrease the extractable tannin content of tea residues by 84% after 4 weeks [49], tannin content of Jatropha biodiesel residues by 85% after 60 days [50], black bean seeds by 65% after 2 weeks, kidney bean seeds by 34% after 2 weeks, and oats seeds by 50% after 2 weeks [51]. This result was explained by the ability of white-rot fungi to produce versatile peroxidase, which can degrade phenolic and nonphenolic compounds [49], and tannase enzyme [50]. Fermentation of grape pomace with both white-rot fungi increased they're mineral content, especially with *G. resinaceum*. This result is consistent with previous studies by Van Kuijk

et al. [42], who found that species belonging to *Ganoderma* genus have a higher capacity to increase the mineral compound of miscanthus than species belonging to *Pleurotus* genus. This increase in mineral nutrients is explained by the release of minerals and inorganic matter, and the losses of organic matter to CO_2 during fungal growth [49]. The high increase of mineral compound of fermented grape pomace requires a change in mineral supplementation for ruminant diets. This strategy also improved the non-fibrous carbohydrates of grape pomace only after 4 weeks of fermentation. This result is consistent with some previous study which noted significant increase on non-fibrous carbohydrates of ryegrass fescue hay fermented with *P. ostreatus* for 2 weeks [52] and corn stover fermented with *P. ostreatus* for 3 weeks [53]. This enhancement on non-fibrous carbohydrates can increase the palatability of feed, improve their performance of ruminants and reduce enteric emission of methane and carbon dioxide [48].

3.2 Rumen fermentation characteristics

In vitro rumen fermentation technique based on gas production is a fast and effective method widely used to study the nutritional value of feeds under simulated rumen conditions [20, 49, 53–55]. The rumen fermentation characteristics of untreated grape pomace are shown in Table 2. Despite the high efficiency of the rumen digestive system in converting fiber biomass into metabolizable energy, about 60% of the dry matter and 80% of the fiber of grape pomace pass through the digestive tract without being digested. In addition, their metabolizable energy is insufficient to meet the metabolizable energy required for maintenance of sheep [56, 57].

Bioconversion of grape pomace with both white-rot fungi in both periods improved rumen fermentation, which

Fig. 1 Gas profile of grape pomace treated with white-rot fungi
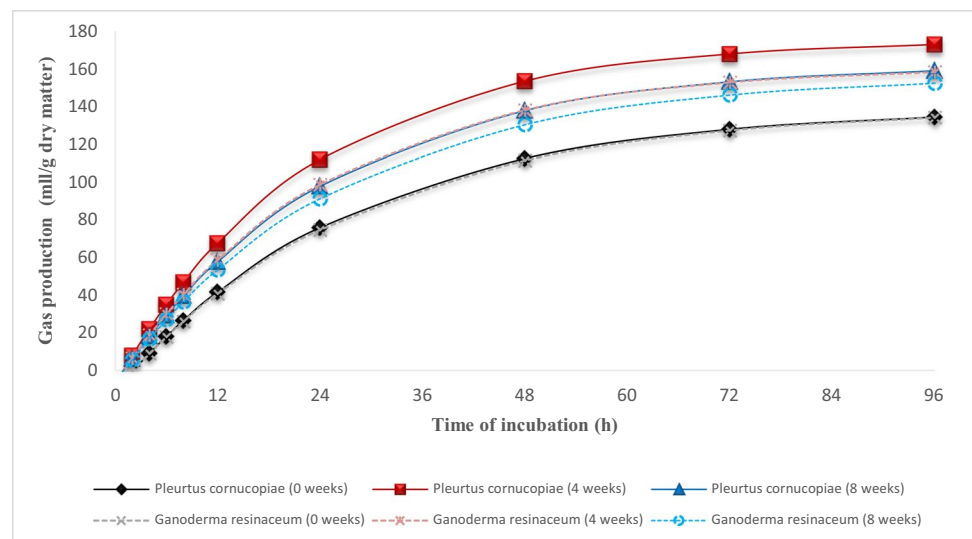
increased asymptotic gas production, accelerated constant gas production rate and shortened time of onset of rumen fermentation. The highest yield was obtained in grape pomace fermented with *P. cornucopiae* for 4 weeks. This improvement is explained by the ability of these white-rot fungi to reduce anti-nutritional compounds namely condensed tannin, which allows better environmental conditions for the proliferation of the rumen microbiota [41] and to decrease lignin content, which allow better accessibility of the rumen microbiota to attack more and faster carbohydrates [14]. This improvement could also be related to the potential of white-rot fungi to eliminate silica bodies, which increase the surface of enzymatic attack and adhesion of rumen microbiota, as shown by Nur-Nazratu et al. [19] on oil palm empty fruit bunch fermented with *G. lucidum,* to the capacity of fungi to reduce the crystallinity of cellulose, as shown by Palangi et al. [55] on cultivation substrate with *Agaricu bisporus* and to the ability of white-rot fungi to produce exogenous lignolytic enzymes that act in synergy with the rumen microbiota and endogenous enzymes in the rumen as shown by Sridhar et al. [58] on ragi straw treated with exogenous lignolytic enzymes from *G. lucidium*. This biological treatment also improved the ruminal digestibility of dry matter and fiber of grape pomace. The achieved results in the present work were significantly better since Mhlongo et al. [14] used 20% to 50% of *P. ostreatus* as inoculum, which could interfere with the substrate composition. This bioprocess also increased the metabolizable energy of grape pomace (Table 2). To our knowledge, this is the first study that demonstrated the ability of white-rot fungi to improve the metabolizable energy of grape pomace. This result was consistent with those reported by Olagunju et al. [53] who noted an increase in metabolizable energy, net energy for maintenance, net energy of growth and net energy of lactation of corn stover fermented with *P. ostreatus*. This

bioprocess also improved the concentration of volatile fatty acids in the rumen. A comparable effect was noted by Datsomor et al. [54] on rice straw fermented with *P. ostreatus* for 30 days. This increase in the concentration of volatile fatty acids favorized proliferation of rumen microbiota [59] and provided additional energy for ruminants [60]. The optimal solid state fermentation duration seems to be around 4 weeks (Table 2, Fig. 1). The reduction of the positive effect of this bioprocess with longer fermentation duration could be explained by the production of antimicrobial compounds by the white-rot fungi when the fermentation colonization lasts longer [18].

This study also showed that fermented grape pomace with both white-rot fungi increased rumen ammonia-nitrogen concentration. Khonkhaeng and Cherdthong [61] found a comparable result with corn residue fermented with *P. ostreatus.* This improvement could be explained by the increase in crude protein in biomass fermented with white-rot fungi and may be attributed to better protein degradation in the rumen [62]. The increase in the concentration of ammonia-nitrogen and volatile fatty acids in the rumen seemed enhance the ability of rumen microbiota to synthesis more microbial crude protein. This was consistent with a previous study by Olagunju et al. [53] in which corn stover was incubated with *P. ostreatus*. The synthesized microbial crude protein has the potential to meet 70% to 100% of the protein requirements of ruminants and replace some dietary protein, resulting in lower cost-effective production [60, 63]. Fermented grape pomace with *P. cornucopiae* for 4 weeks slight decreased rumen pH from 6.47 to 6.37 compared to control grape pomace, but it is still within the optimal pH range (6.3 to 6.8) that favors normal proliferation of rumen microbiota and digestive activity [64]. This is consistent with the results of Yan et al. [49] using *Phanerochaete chrysosporium* on white tea residues.

4 Conclusion

Grape pomace is an abundant waste worldwide and represents a serious environmental problem. It is rarely used in ruminant feeding because it contains high levels of lignin and antinutritional compounds that limit rumen fermentation, digestibility, metabolizable energy, and microbial crude protein synthesis. Pretreatment of this abundant waste with white-rot fungi can be used as a simple and effective strategy to not only reduce lignin, but also partially reduce condensed tannin and improve crude protein content. Consequently, this strategy improves the rumen fermentation process and rumen digestibility of dry matter and fiber. This improvement provides more energy that can be metabolized by ruminants and a suitable substrate for the rumen microbiota to produce microbial crude protein. However, increasing the duration of colonization decreases digestibility, usable energy, and microbial crude protein synthesis, underscoring the need for precise management during the fermentation process. It is important to note that although *P. cornucopiae* and *G. resinaceum* showed similarities in increasing protein content and reducing tannins, the highest selectivity in lignin degradation with *P. cornucopiae* requires further investigation. Nevertheless, *P. cornucopiae* proves to be a favorable choice for optimizing the nutritional value of grape pomace maximizing the benefits of this waste utilization strategy.

By conducting a new study that examines the potential benefits of this process in more detail, focusing on the profile of amino acids produced and synthesized by microbial protein, we can provide additional evidence for the importance of this strategy. This study would provide valuable insight into the nutritional value and amino acid composition of the microbial protein produced. Knowledge of the amino acid profile would allow us to determine the suitability of this protein source to meet the specific nutritional needs of ruminants and optimize their performance. This strategy should be investigated in future in vivo feeding trials to study the effects of solid-state fermented grape pomace with *P. cornucopiae* on growth performance and health of ruminants.

Abbreviations *ADF*: acid detergent fiber; *ADL*: acid detergent lignin; *B*: amount of rumen fermentation; *C*: rate of rumen fermentation; *CE*: cellulose; *CP*: crude protein; *CT*: condensed tannin; *DMD*: dry matter digestibility; *EE*: ether extract; *HC*: hemicellulose; *Lag*: time of the onset of rumen fermentation; *MCP*: microbial crude protein; *ME*: metabolizable energy; *NDF*: neutral detergent fiber; *NDFD*: neutral detergent fiber digestibility; *NFC*: non-fiber carbohydrate; $NH_3\text{-}N$: rumen ammonia-nitrogen; *SEM*: standard error of means; *VFA*: volatile fatty acids

Author contribution Conceptualization: KA, TN, and AJ; methodology: KA and AJ; format analyses and investigation: KA, SB, and OA; funding acquisition: TN and AJ; writing original draft: KA; writing—review and editing: KA and AJ; All authors read and approved the final manuscript.

Data availability The datasets and materials used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval The article does not contain any studies with human participants. It also does not perform experiments directly on animals, So, this experience not need ethics statement.

Competing interests The authors declare no competing interests.

References

1. FAO/OIV (2021) Annual Assessment of the World Vine and Wine Sector in 2021 International Organisation of Vine and Wine Intergovernmental Organisation. https://www.oiv.int/sites/default/files/documents/OIV_Annual_Assessment_of_the_World_Vine_and_Wine_Sector_in_2021.pdf. Accessed 10 Jun 2023
2. Toscano G, Riva G, Duca D, Pedretti EF, Corinaldesi F, Rossini G (2013) Analysis of the characteristics of the residues of the wine production chain finalized to their industrial and energy recovery. Biomass and Bioenergy 55:260–267. https://doi.org/10.1016/j.biombioe.2013.02.015
3. Dwyer K, Hosseinian F, Rod M (2014) The market potential of grape waste alternatives. J Food Res 3(2):91–106. https://doi.org/10.5539/jfr.v3n2p91
4. Khiari B, Jeguirim M (2018) Pyrolysis of grape marc from Tunisian wine industry: feedstock characterization, thermal degradation and kinetic analysis. Energies 11(4):730. https://doi.org/10.3390/en11040730
5. Moutinho J, Gouvinhas I, Domínguez-Perles R, Barros A (2023) Optimization of the extraction methodology of grape pomace polyphenols for food applications. Molecules 28(9):3885. https://doi.org/10.3390/molecules28093885
6. Megías-Pérez R, Ferreira-Lazarte A, Villamiel M (2023) Valorization of grape pomace as a renewable source of techno-functional and antioxidant pectins. Antioxidants 12(4):957. https://doi.org/10.3390/antiox12040957
7. Kokkinomagoulos E, Kandylis P (2023) Grape pomace, an undervalued by-product: industrial reutilization within a circular economy vision. Rev Environ Sci Biotechnol 22:739–773. https://doi.org/10.1007/s11157-023-09665-0
8. Erskine E, Ozkan G, Lu B, Capanoglu E (2023) Effects of fermentation process on the antioxidant capacity of fruit byproducts. ACS Omega 8(5):4543–4553. https://doi.org/10.1021/acsomega.2c07602
9. Bennato F, Martino C, Ianni A, Giannone C, Martino G (2023) Dietary grape pomace supplementation in lambs affects the meat fatty acid composition, volatile profiles and oxidative stability. Foods 12(6):1257. https://doi.org/10.3390/foods12061257
10. Bennato F, Ianni A, Florio M, Grotta L, Pomilio F, Saletti MA, Martino G (2022) Nutritional properties of milk from dairy ewes

fed with a diet containing grape pomace. Foods 11(13):1878. https://doi.org/10.3390/foods11131878

11. Zhao J, Jin Y, Du M, Liu W, Ren Y, Zhang C, Zhang J (2017) The effect of dietary grape pomace supplementation on epididymal sperm quality and testicular antioxidant ability in ram lambs. Theriogenology 97:50–56. https://doi.org/10.1016/j.theriogenology.2017.04.010

12. Baumgärtel T, Kluth H, Epperlein K, Rodehutscord M (2007) A note on digestibility and energy value for sheep of different grape pomace. Small Rumin Res 67(2-3):302–306. https://doi.org/10.1016/j.smallrumres.2005.11.002

13. Abarghuei MJ, Rouzbehan Y, Alipour D (2010) The influence of the grape pomace on the ruminal parameters of sheep. Livest Sci 132(1-3):73–79. https://doi.org/10.1016/j.livsci.2010.05.002

14. Mhlongo G, Mnisi CM, Mlambo V (2021) Cultivating oyster mushrooms on red grape pomace waste enhances potential nutritional value of the spent substrate for ruminants. PLoS One 16(2):e0246992. https://doi.org/10.1371/journal.pone.0246992

15. Adesogan AT, Arriola KG, Jiang Y, Oyebade A, Paula EM, Pech-Cervantes AA, Romero JJ, Ferraretto LF, Vyas D (2019) Symposium review: technologies for improving fibre utilization. J Dairy Sci 102:5726–5755. https://doi.org/10.3168/jds.2018-15334

16. Dashora K, Gattupalli M, Tripathi GD, Javed Z, Singh S, Tuohy M, Sarangi PK, Diwan D, Singh HB, Gupta VK (2023) Fungal assisted valorisation of polymeric lignin: mechanism, enzymes and perspectives. Catalysts 13(1):149. https://doi.org/10.3390/catal13010149

17. Ariff INM, Bahrin EK, Ramli N, Abd-Aziz S (2019) Direct use of spent mushroom substrate from *Pleurotus pulmonarius* as a readily delignified feedstock for cellulase production. Waste Biomass Valorization 10:839–850. https://doi.org/10.1007/s12649-017-0106-8

18. Rahman MM, Lourenço M, Hassim HA, Baars JJ, Sonnenberg AS, Cone JW, De Boever J, Fievez V (2011) Improving ruminal degradability of oil palm fronds using white rot fungi. Anim Feed Sci Technol 169(3-4):157–166. https://doi.org/10.1016/j.anifeedsci.2011.06.014

19. Nur-Nazratul FMY, Rakib MRM, Zailan MZ, Yaakub H (2021) Enhancing *in vitro* ruminal digestibility of oil palm empty fruit bunch by biological pre-treatment with Ganoderma lucidum fungal culture. PLoS One 16(9):e0258065. https://doi.org/10.1371/journal.pone.0258065

20. Sufyan A, Ahmad N, Shahzad F, Embaby MG, AbuGhazaleh A, Khan NA (2022) Improving the nutritional value and digestibility of wheat straw, rice straw, and corn cob through solid state fermentation using different Pleurotus species. J Sci Food Agric 102(6):2445–2453. https://doi.org/10.1002/jsfa.11584

21. Xu LN, Guo S, Zhang S (2018) Effects of solid-state fermentation with three higher fungi on the total phenol contents and antioxidant properties of diverse cereal grains. FEMS Microbiol Lett 365(16):fny163. https://doi.org/10.1093/femsle/fny163

22. Gebru YA, Sbhatu DB (2020) Effects of fungi-mediated solid-state fermentation on phenolic contents and antioxidant activity of brown and white teff (*Eragrostis tef* (Zucc.) Trotter) grains. J Food Qual 2020:1–11. https://doi.org/10.1155/2020/8819555

23. Sánchez-García J, Asensio-Grau A, García-Hernández J, Heredia A, Andrés A (2022) Nutritional and antioxidant changes in lentils and quinoa through fungal solid-state fermentation with *Pleurotus ostreatus*. Bioresour Bioprocess 9(1):1–12. https://doi.org/10.1186/s40643-022-00542-2

24. Vorlaphim T, Paengkoum P, Purba RAP, Yuangklang C, Paengkoum S, Schonewille JT (2021) Treatment of rice stubble with *Pleurotus ostreatus* and urea improves the growth performance in slow-growing goats. Animals 11(4):1053. https://doi.org/10.3390/ani11041053

25. Fan GJ, Chen MH, Lee CF, Yu B, Lee TT (2022) Effects of rice straw fermented with spent *Pleurotus sajor-caju* mushroom substrates on milking performance in Alpine dairy goats. Anim Biosci 35(7):999. https://doi.org/10.5713/ab.21.0340

26. Martens SD, Wildner V, Zeyner A, Steinhöfel O (2023) *In vitro* ruminal degradability of wheat straw cultivated with white-rot fungi adapted to mushroom farming conditions. Sci Rep 13(1):7794. https://doi.org/10.1038/s41598-023-34747-y

27. Association of Official Chemists Analytical Chemists (2016) Official Methodes of analysis of AOAC international, 20th edn. AOAC International, Gaithersburg, MD, USA

28. Van Soest PV, Robertson JB, Lewis BA (1991) Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J Dairy Sci 74:3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

29. Makkar HPS (2000) Quantification of tannins in tree foliage. IAEA. A laboratory manual FAO/IAEA Working Document,Vienna, Austria.

30. Theodorou MK, Williams BA, Dhanoa MS, McAllan AB, France JA (1994) simple gas production method using a pressure transducer to determine the fermentation kinetics of ruminant feeds. Anim Feed Sci Technol 48:185–197. https://doi.org/10.1016/0377-8401(94)90171-6

31. Menke KH, Steingass H (1988) Estimation of the energetic feed value obtained from chemical analysis and *in vitro* gas production using rumen fluid. Anim Res Dev 28:7–55

32. Mauricio RM, Mould FL, Dhanoa MS, Owen E, Channa KS, Theodorou MK (1999) A semi-automated *in vitro* gas production technique for ruminant feedstuff evaluation. Anim Feed Sci Technol 79(4):321–330. https://doi.org/10.1016/S0377-8401(99)00033-4

33. France J, Dijkstra J, Dhanoa MS, Lopez S, Bannink A (2000) Estimating the extent of degradation of ruminant feeds from a description of their gas production profiles observed *in vitro*: derivation of models and other mathematical considerations. Br J Nutr 83(2):143–150. https://doi.org/10.1017/S0007114500000180

34. SAS Institute Inc (2011) SAS/STAT 9.3, User's Guide. SAS Institute Inc, Cary, NC, USA

35. Broderick GA, Kang JH (1980) Automated simultaneous determination of ammonia and total amino acids in ruminal fluid and *in vitro* media. J Dairy Sci 63:64–75. https://doi.org/10.3168/jds.S0022-0302(80)82888-8

36. Getachew G, Blümmel M, Makkar HPS, Becker K (1998) *In vitro* gas measuring techniques for assessment of nutritional quality of feeds: a review. Anim Feed Sci Technol 72(3-4):261–281. https://doi.org/10.1016/S0377-8401(97)00189-2

37. Blümmel M, Makkar HPS, Becker K (1997) *In vitro* gas production: a technique revisited. J Anim Physiol An N 77:24–34. https://doi.org/10.1111/j.1439-0396.1997.tb00734.x

38. Abid K, Jabri J, Beckers Y, Yaich H, Malek A, Rekhis J, Kamoun M (2019) Effects of exogenous fibrolytic enzymes on the ruminal fermentation of agro-industrial by-products. S Afr J Anim Sci 49(4):612–618. https://doi.org/10.4314/sajas.v49i4.2

39. Van Soest PV (1994) Nutritional Ecology of the Ruminant, 2nd edn. Comstock, Ithaca, NY, USA

40. Nasehi M, Torbatinejad NM, Zerehdaran S, Safaie AR (2017) Effect of solid-state fermentation by oyster mushroom (*Pleurotus florida*) on nutritive value of some agro by-products. J Appl Anim Res 45(1):221–226. https://doi.org/10.1080/09712119.2016.1150850

41. Besharati M, Maggiolino A, Palangi V, Kaya A, Jabbar M, Eseceli H, De Palo P, Lorenzo JM (2022) Tannin in Ruminant Nutrition. Molecules 27(23):8273. https://doi.org/10.3390/molecules27238273

42. Abid K, Jabri J, Yaich H, Malek A, Rekhis J, Kamoun M (2022) Improving the nutritional value and rumen fermentation characteristics of sesame seed coats through bioconversion approach using exogenous fibrolytic enzymes produced by *Trichoderma*

longibrachiatum. Biomass Convers Biorefin:1–9. https://doi.org/10.1007/s13399-022-03402-3

43. Van Kuijk SJ, Sonnenberg AS, Baars JJ, Hendriks WH, Cone JW (2015) Fungal treatment of lignocellulosic biomass: importance of fungal species, colonization and time on chemical composition and *in vitro* rumen degradability. Anim Feed Sci Technol 209:40–50. https://doi.org/10.1016/j.anifeedsci.2015.07.026

44. Kaal EE, de Jong ED, Field JA (1993) Stimulation of ligninolytic peroxidase activity by nitrogen nutrients in the white rot fungus *Bjerkandera sp*. strain BOS55. Appl Environ Microbiol 59(12):4031–4036. https://doi.org/10.1128/aem.59.12.4031-4036.1993

45. Chen X, Yan F, Liu T, Zhang Y, Li X, Wang M, Zhang C, Xu X, Deng L, Yao J, Wu S (2022) Ruminal microbiota determines the high-fiber utilization of ruminants: evidence from the ruminal microbiota transplant. Microbiol Spectr 10(4):e00446–e00422. https://doi.org/10.1128/spectrum.00446-22

46. Weimer PJ (2022) Degradation of cellulose and hemicellulose by ruminal microorganisms. Microorganisms 10(12):2345. https://doi.org/10.3390/microorganisms10122345

47. Tuyen DV, Phuong HN, Cone JW, Baars JJP, Sonnenberg ASM, Hendriks WH (2013) Effect of fungal treatments of fibrous agricultural by-products on chemical composition and *in vitro* rumen fermentation and methane production. Bioresour Technol 129:256–263. https://doi.org/10.1016/j.biortech.2012.10.128

48. Nayan N, Sonnenberg AS, Hendriks WH, Cone JW (2018) Variation in the solubilization of crude protein in wheat straw by different white-rot fungi. Anim Feed Sci Technol 242:135–143. https://doi.org/10.1016/j.anifeedsci.2018.06.009

49. Yan Q, Lin M, Huang Y, Datsomor O, Wang K, Zhao G (2022) Effects of solid-state fermentation pretreatment with single or dual culture white rot fungi on white tea residue nutrients and *in vitro* rumen fermentation parameters. Fermentation 8(10):557. https://doi.org/10.3390/fermentation8100557

50. da Luz JMR, Paes SA, Torres DP, Nunes MD, da Silva JS, Mantovani HC, Kasuya MCM (2013) Production of edible mushroom and degradation of antinutritional factors in jatropha biodiesel residues. LWT 50(2):575–580. https://doi.org/10.1016/j.lwt.2012.08.006

51. Espinosa-Páez E, Alanis-Guzmán MG, Hernández-Luna CE, Báez-González JG, Amaya-Guerra CA, Andrés-Grau AM (2017) Increasing antioxidant activity and protein digestibility in *Phaseolus vulgaris* and *Avena sativa* by fermentation with the *Pleurotus ostreatus* Fungus. Molecules 22(12):2275. https://doi.org/10.3390/molecules22122275

52. Astudillo-Neira R, Muñoz-Nuñez E, Quiroz-Carreno S, Avila-Stagno J, Alarcon-Enos J (2022) Bioconversion in ryegrass-fescue hay by *pleurotus ostreatus* to increase their nutritional value for ruminant. Agriculture 12(4):534. https://doi.org/10.3390/agriculture12040534

53. Olagunju LK, Isikhuemhen OS, Dele PA, Anike FN, Essick BG, Hol N, Udombang NS, Ike KA, Shaw Y, Brice RM, Orimaye OE, Wuaku M, Anele UY (2023) *Pleurotus ostreatus* can significantly improve the nutritive value of lignocellulosic crop residues. Agriculture 13(6):1161. https://doi.org/10.3390/agriculture13061161

54. Datsomor O, Yan Q, Wang K, Mohamed S, Opoku-Mensah L, Zhao G, Miao L (2022) Effect of ammoniated and/or basidiomycete white-rot fungi treatment on rice straw proximate composition, cell wall component, and *in vitro* rumen fermentation characteristics. Fermentation 8(5):228. https://doi.org/10.3390/fermentation8050228

55. Palangi V, Kaya A, Kaya A, Giannenas I (2022) Ecofriendly usability of mushroom cultivation substrate as a ruminant feed: anaerobic digestion using gas production techniques. Animals 12(12):1583. https://doi.org/10.3390/ani12121583

56. Al Jassim RAM, Hassan SA, Al-Ani AN (1996) Metabolizable energy requirements for maintenance and growth of Awassi lambs. Small Rumin Res 20(3):239–245. https://doi.org/10.1016/0921-4488(95)00775-X

57. Dawson LER, Steen RWJ (1998) Estimation of maintenance energy requirements of beef cattle and sheep. J Agric Sci 131(4):477–485. https://doi.org/10.1017/S0021859698005942

58. Sridhar M, Bhatta R, Dhali A, Saravanan M, Pradeep V, Thammaiah V (2015) Effect of exogenous lignolytic enzyme-treated ragi straw on DM intake, digestibility, rumen fermentation and rumen enzymes in sheep. Indian J Anim Sci 85(9):1012–1016

59. Villalba JJ, Ates S, MacAdam JW (2021) Non-fiber carbohydrates in forages and their influence on beef production systems. Front Sustain Food Syst 5:566338. https://doi.org/10.3389/fsufs.2021.566338

60. Thirumalesh T, Krishnamoorthy U (2013) Rumen microbial biomass synthesis and its importance in ruminant production. Int J Livest Res 3(2):5–26. https://doi.org/10.5455/ijlr.20130502081346

61. Khonkhaeng B, Cherdthong A (2020) Improving nutritive value of purple field corn residue and rice straw by culturing with white-rot fungi. J Fungi 6(2):69. https://doi.org/10.3390/jof6020069

62. Hristov AN, Etter RP, Ropp JK, Grandeen KL (2004) Effect of dietary crude protein level and degradability on ruminal fermentation and nitrogen utilization in lactating dairy cows. J Anim Sci 82(11):3219–3229. https://doi.org/10.2527/2004.82113219x

63. Zurak D, Kljak K, Aladrović J (2023) Metabolism and utilisation of non-protein nitrogen compounds in ruminants: a review. J Cent Eur Agric 24(1):1–14. https://doi.org/10.5513/JCEA01/24.1.3645

64. Chanjula P, Wungsintaweekul J, Chiarawipa R, Rugkong A, Khonkhaeng B, Suntara C, Cherdthong A (2022) Effect of feed supplement containing dried kratom leaves on apparent digestibility, rumen fermentation, serum antioxidants, hematology, and nitrogen balance in goats. Fermentation 8(3):131. https://doi.org/10.3390/fermentation8030131