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# Oyster mushroom bioprocessing enhances the nutritional value of olive pomace for ruminants

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## Abstract

Incorporating olive pomace (OP) into ruminant feed can alleviate the environmental impact of OP disposal, minimize food–feed competition, and bolster food security. However, high fibre and low crude protein (CP) reduce its nutritional value for ruminants. The study assessed the effect of oyster mushrooms (OYM) on the nutritive value of OP. To this end, chemical composition and *in vitro* ruminal fermentation parameters of spent OP substrate were evaluated. The OYM was spawned on 200 g OP at the rate of 0 (OP0), 10 (OP10), 20 (OP20), 30 (OP30), 40 (OP40), and 50% w/w (OP50) and cultivated for 35 d with 10 replicate pots per spawning rate. Dry matter, organic matter, CP, and ash increased linearly, whereas neutral detergent fibre and crude fat declined linearly. *In vitro* cumulative gas production at 12, 24, 36, 48, 72, and 96 h showed a positive quadratic response. Spawning rates linearly enhanced the immediately fermentable fraction (*a*) and rate of gas production from the slowly fermentable fraction (*c*), while the partitioning factor increased linearly. Positive quadratic responses were noted for the slowly fermentable fraction, potential gas production, effective gas production, and *in vitro* organic matter degradation at 96 h of ruminal incubation. In conclusion, OYM enhanced the nutritive value of OP by reducing fibre content, *a*, and *c* fractions while improving CP content and fermentation efficiency. This approach facilitates the bioconversion of OP into sustainable ruminant feed, leading to lower feed costs, addressing waste disposal issues, and creating supplementary income for the olive oil industry.

**Keywords:** bioconversion, chemical composition, *Olea europea*, *Pleurotus ostreatus*, waste disposal, ruminal fermentation

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## Introduction

Ruminants are vital in addressing nutritional and socioeconomic needs in semi-arid and arid areas (Leite *et al.*, 2021). However, to ensure economic, environmental, and social sustainability, their diets should include feedstuffs with no direct food value for humans (Mhlongo *et al.*, 2021), such as olive (*Olea europea*) pomace (OP) produced from olive oil extraction. Currently, large quantities of OP are discarded in landfills as waste (Albini *et al.*, 2023) to the detriment of the environment (Alkhalidi *et al.*, 2023; Díaz-Perete *et al.*, 2023). This includes disruptions in plant development and eutrophication. Therefore, it is crucial to explore alternative applications of OP that can enhance its utility while minimizing its adverse environmental effects.

Therefore, the identification of alternative uses for OP becomes essential in bolstering the environmental sustainability of the olive oil production sector. This by-product contains protein, fibre,

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vitamin E, minerals, polyunsaturated fatty acids (PUFAs), phenolics, oleuropein, and flavonoids (Nunes *et al.*, 2018; Difonzo *et al.*, 2021; Hlatshwayo *et al.*, 2023), which can be utilized to enhance the health and performance of ruminants. Hlatshwayo *et al.* (2023) reported that OP contains 46 g/kg DM crude protein and 296 g/kg DM crude fats, suggesting its potential as a low-cost and sustainable alternative to costly conventional energy sources such as maize in ruminant rations. However, high fibre (426 g/kg DM) content in OP (Fathy *et al.*, 2018), may decrease fermentation efficiency leading to poor performance of ruminants when fed at high levels. Abbeddou *et al.* (2011) found lower OM and NDF digestibility (47.8 and 42.7%, respectively) in Awassi sheep fed OP compared to a barley–wheat bran mixture (66.3 and 56.3%, respectively). Bermúdez-Oria *et al.* (2021) showed that beneficial phenols and bioactive compounds in olive pomace (OP) were chemically linked to non-starch polysaccharides (NSPs), which influenced the physical characteristics of the digesta in the rumen. Soluble NSPs can increase the viscosity of the rumen contents, which may slow down the passage rate of digesta and reduce overall feed intake and digestion. However, restrictions in ruminant digestion affect the utilisation and availability of these compounds. Therefore, it is crucial to explore methods, such as bioprocessing of OP with oyster mushrooms (*Pleurotus ostreatus*; OYM), to enhance fibre degradation.

Fungal bioprocessing is commonly considered an economically and environmentally viable approach to upcycle substrates rich in lignin (Fathy *et al.*, 2018). Generally, mushrooms thrive on organic waste, acting as key decomposers that transform decaying matter into nutrients necessary for their development (Niego *et al.*, 2023). Before assimilating these nutrients, their mycelia release digestive enzymes to break down lignin–cellulose into smaller particles. Cultivating OYM on red grape pomace substrate enhances the nutritional quality of the spent substrate by decreasing fibre content and elevating crude protein content (Mhlongo *et al.*, 2021). Improving OP digestibility could yield meat with health-promoting attributes for humans and simultaneously address environmental concerns linked to OP disposal and enteric methane emission. The objective of the study was to assess the impact of various OYM spawning rates on the chemical composition and *in vitro* ruminal fermentation parameters of OP. We hypothesized that cultivating OYM on OP would decrease the fibre fraction and increase the crude protein content of the spent substrate, potentially enhancing its ruminal fermentation compared to untreated substrates.

## Materials and Methods

This investigation was conducted between June and October 2023 at the North-West University, Molelwane Research Farm, South Africa. Fresh OP was obtained from Stoneberg Farm Holdings (Pty) Ltd (Western Cape, South Africa) and processed as described by Hlatshwayo *et al.* (2023). The OYM spawn was acquired from Eco-Argo Enterprise (PTY) LTD (Nelspruit, South Africa).

The OP substrate was sterilized at 121 °C for 1 h at 100 kPa in an autoclave (BKQ-B120II; BIOBASE group, Shandong, China) and cooled to room temperature (20–25 °C), as described by Tuyen *et al.* (2012). An amount of 200 g of OP substrate was allocated to each of the 60 pots (223 $cm^3$ each), serving as experimental units. The OP substrates in pots were randomly inoculated with OYM spawn at 0 (OP0), 10 (OP10), 20 (OP20), 30 (OP30), 40 (OP40), or 50% w/w (OP50), with each spawning rate replicated 10 times. The pots were kept at room temperature (20–25 °C) and 70–80% relative humidity throughout the 35-d experiment. A thermo-hydrometer (HTC-1, Xuzhou Sanhe Automatic Control Equipment Co., Ltd, China) was used to monitor substrate humidity every 2 d. Inoculated OP substrates and control (OP0) were sprinkled with water using a 125-ml spray bottle every 2 d and black plastic bags were used to cover the pots to maintain the moisture levels required for mushroom spore reproduction. Uninoculated OP substrate in pots was treated similarly. After a 35-d incubation, 125 g of spent mushroom substrate (SMS) was sampled from each pot and mycelia was removed manually before the sample was oven-dried at 60 °C until a constant weight was reached. The dried SMS was milled through a 1-mm sieve (Polymix PX-MFC 90 D, Switzerland) and stored in labelled bottles until required for chemical analysis and *in vitro* ruminal fermentation.

The SMS was analysed for dry matter (DM; method no. 930.15), organic matter (OM; method no. 942.05), and crude protein (CP; method no. 984.13) using the Association of Official Analytical Chemists (2005) methods. Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using an ANKOM$^{200}$ Fibre Analyser (ANKOM Technology, New York) by refluxing 0.45–0.5 g of the milled SMS with a neutral detergent and acid detergent solutions for 60 min and 75 min, respectively, as described by Van Soest *et al.* (1991). The NDF procedure involved the utilization of alpha-amylase as per the manufacturer's guidelines. To determine the acid detergent lignin (ADL), the residues (in ANKOM F57 bags) from the ADF determination were then soaked in 72% $H_2SO_4$ for 3 h to dissolve the cellulose in the samples and oven-dried overnight. Following the drying of 2 g of the sample on filter paper at 100 °C for 2 h, it was subjected to ether extraction in a Soxhlet extractor (ANKOM $^{XT15}$ Extractor,

ANKOM Technology, NY, USA) at 80 °C for 8 h. The extracted ether was then dried in a solvent flask at 100 °C for 3 h, followed by cooling in a desiccator for 40 min.

The Reading Pressure Technique (Mauricio *et al.*, 1999) was used to determine the *in vitro* ruminal fermentation characteristics of OP. Rumen inoculum was obtained in the morning from a rumen-fistulated, 600-kg Bonsmara cow that was cared for according to protocols authorized (NWU-00126-13-A9) by the North-West University's Animal Production Research Ethics Committee. Rumen fluid was collected into a pre-warmed flask, then blended, and filtered through a two-layered muslin cloth in the laboratory. To replicate the anaerobic rumen environment, filtered rumen fluid was kept at 39 °C with continuous $CO_2$ gas purging until used for inoculation. Ground SMS samples (1 ± 0.001 g each) were weighed into 125-ml glass serum bottles to which 90 ml of ANKOM buffer solution was added. The contents were purged with $CO_2$ gas and the bottles were then sealed with rubber stoppers before being placed in a 39 °C incubator overnight. In the morning, 10 ml of rumen fluid was injected into each serum bottle using a syringe followed by incubation at 39 °C for 96 h. The headspace gas pressure was determined at 0, 2, 4, 6, 8, 12, 24, 36, 48, 72, and 96-h intervals using a 23-gauge needle connected to a pressure transducer (model PX4200-015GI, Omega Engineering, Inc., Laval, QC, Canada).

The pressure transducer recorded peak gas pressure (psi), which was then converted to gas volume (mL) using the following site-specific equation previously generated through a calibration process:

$$y = 0.034x^2 + 6.2325x + 1.8143 \quad (1)$$

where $y$ = gas volume (mL) and $x$ = measured gas pressure (psi).

The gas production rate per period was determined by dividing the volume of gas produced during each period by the corresponding number of hours. Cumulative gas production data were modelled using the Ørskov & McDonald (1979) non-linear model:

$$Y = a + b\left(1 - e^{-c(t)}\right) \quad (2)$$

where $a$ = the immediately fermentable fraction, $b$ = the slowly fermentable fraction, $c$ = the rate of constant from the slowly fermentable fraction, $b$.

Gas production potential (*Pgas*) was determined as the sum of fractions $a$ and $b$, whereas effective gas production (*Egas*) was computed using the formula:

$$Egas = a + \frac{b \times c}{k+c} \quad (3)$$

with $k$ representing a 2% hourly rumen outflow.

*In vitro* ruminal organic matter degradability (*iv*OMD) was assessed 96 h post-inoculation by filtering fermentation residue through glass-sintered crucibles (100–160-µm porosity, Pyrex, Stone, UK). Partitioning factors (PF) were computed as the ratio of cumulative gas production (mL) at 96 h post-incubation to *iv*OMD (mg).

Response surface regression analysis (Proc RSREG; SAS 2010) was used to analyse linear and quadratic responses in the OP's chemical components and *in vitro* ruminal fermentation parameters in response to OYM spawning rates. Chemical composition and *in vitro* ruminal fermentation data were analysed using one-way ANOVA (PROC GLM; SAS 2010) in a completely randomized design, with OYM spawn rate as the sole factor. Significance was declared at $P$ <0.05 for all statistical tests, and least-squares means were compared using the probability of difference option in SAS.

## Results and Discussion

The chemical composition of olive pomace spent OYM substrate is shown in Table 1. Response surface regression analysis revealed linear increases for DM [$y$ = 0.266 (0.217) $x$ + 986 (2.34); $R^2$ = 0.077; $P$ = 0.049], CP [$y$ = 0.16 (±0.11) $x$ + 61.9 (±1.18); $R^2$ = 0.712; $P$ = <0.0001], ADF [$y$ = 0.414 (±1.10) $x$ + 354 (±11.9); $R^2$ = 0.263; $P$ = 0.0001], ADL [$y$ = 0.125 (±0.74) $x$ + 223 (±7.95); $R^2$ = 0.342; $P$ = <.0001], ash [$y$ = 0.606 (±0.11) $x$ + 17.0 (±1.21); $R^2$ = 0.897; $P$ = <0.0001], and linear decreases for OM [$y$ = 969 (±2.31) – 0.34 (±0.21) $x$; $R^2$ = 0.620; $P$ = <0.0001], NDF [$y$ = 532 (±10.2) – 0.738 (±0.94) $x$; $R^2$ = 0.419; $P$ = <0.0001] and crude fat [$y$ = 349 (±7.52) – 2.21 (±0.73) $x$ +; $R^2$ = 0.589; $P$ = <0.0001] in response to OYM spawning rates. Except for DM and cellulose, spawning rates substantially affected all the evaluated chemical components. The control group (OP0) had a higher ($P$ <0.05) OM (969 g/kg

DM) than OP30, OP40, and OP50, but was similar to OP10 and OP20 ($P$ >0.05). OP40 and OP50 promoted the highest ($P$ <0.05) CP followed by OP10, OP20, and OP30, which were similar, whereas OP0 promoted the lowest value. OP0 and OP10 had similar CP mean values. OP0 had a higher ($P$ <0.05) NDF and ADF composition than OP50, which was similar ($P$ >0.05) to OP10, OP20, and OP30. OP40 and OP50 had similar lower ($P$ <0.05) ADL values than OP0, OP10, OP20, and OP30, which were similar ($P$ >0.05). The ADL composition of OP20 and OP40 were similar. OP0 and OP10 promoted comparably higher ($P$ <0.05) CF content than OP30, OP40, and OP50, which were similar ($P$ >0.05). OP0 had the lowest ($P$ <0.05) ash content, followed by OP10, OP20, OP30, and OP40, whereas OP50 had the highest value.

**Table 1** Chemical composition (g/kg DM, unless indicated otherwise) of olive pomace spent substrates

| | Substrates[1] | | | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Parameters[2] | OP0 | OP10 | OP20 | OP30 | OP40 | OP50 | SEM[3] | $P$-$_{GLM}$ | $P$-$_{Linear}$ | $P$-$_{Quadratic}$ |
| Dry matter (g/kg) | 986 | 989 | 989 | 994 | 992 | 992 | 2.44 | 0.187 | 0.049 | 0.488 |
| Organic matter | 969$^{d}$ | 965$^{cd}$ | 961$^{cd}$ | 956$^{bc}$ | 949$^{ab}$ | 942$^{a}$ | 2.43 | <.0001 | <0.0001 | 0.301 |
| Crude protein | 61.4$^{a}$ | 65.6$^{ab}$ | 67.6$^{b}$ | 67.0$^{b}$ | 76.5$^{c}$ | 79.9$^{c}$ | 1.21 | <.0001 | <0.0001 | 0.059 |
| NDF | 530$^{c}$ | 526$^{c}$ | 501$^{bc}$ | 507$^{bc}$ | 468$^{ab}$ | 453$^{a}$ | 10.4 | 0.0001 | <0.0001 | 0.338 |
| ADF | 356$^{c}$ | 352$^{bc}$ | 346$^{bc}$ | 352$^{bc}$ | 305$^{ab}$ | 297$^{a}$ | 12.2 | 0.001 | 0.0001 | 0.121 |
| ADL | 226$^{c}$ | 222$^{c}$ | 208$^{bc}$ | 220$^{c}$ | 184$^{ab}$ | 173$^{a}$ | 8.03 | 0.0001 | <0.0001 | 0.108 |
| Crude fat | 335$^{c}$ | 348$^{c}$ | 303$^{b}$ | 285$^{ab}$ | 278$^{ab}$ | 269$^{a}$ | 6.24 | <.0001 | <0.0001 | 0.346 |
| Cellulose | 132 | 131 | 138 | 132 | 121 | 124 | 7.33 | 0.546 | 0.198 | 0.392 |
| Hemicellulose | 176 | 174 | 155 | 155 | 163 | 157 | 9.53 | 0.465 | 0.131 | 0.334 |
| Ash | 16.5$^{a}$ | 24.0$^{b}$ | 27.8$^{b}$ | 38.6$^{c}$ | 43.1$^{c}$ | 50.7$^{d}$ | 1.23 | 0.0001 | <0.0001 | 0.523 |

$^{abcd}$In a row, different superscripts signify significant differences ($P$ <0.05) between spawning rate means
[1]Substrates: OP0 = uninoculated olive pomace, OP10–50 = olive pomace inoculated with 10–50% oyster mushroom spawn
[2]Parameters: NDF = neutral detergent fibre, ADF = acid detergent fibre, ADL = acid detergent lignin
[3]SEM = standard error mean

The oyster mushroom exhibits a potent fibrolytic mechanism (Illuri *et al.,* 2021), which is supported by diverse enzyme activity depending on the complexity of the associated substrate (Kumla *et al.,* 2020). The observed decrease in DM and OM content of OP in response to OYM in this study corroborates earlier reports for red grape pomace (Mhlongo *et al.*, 2021) and corn stover (Lorenzana-Moreno *et al.*, 2023) when bioprocessed with 500 and 50 g/kg OYM, respectively. The decrease in DM and OM is a result of the consumption of substrate carbohydrates in the cell wall, indicating that this decline correlates with the extent of mycelial development (Muswati *et al.*, 2021; Zakil *et al.*, 2022). Higher CP content in OYM-treated OP can be attributed to the reduction in the carbohydrate fraction due to fungal hydrolysis (Uwineza *et al.*, 2023) and its subsequent use as a carbon source to generate protein-rich fungal biomass (Fawzy & Gomaa, 2020). Furthermore, OYM may boost CP by producing proteins from nitrates and amines in fermentation substrates (Walker & White, 2017; De *et al.*, 2023). Consistent with our results, Mhlongo *et al.* (2021), Abid *et al.* (2023), and Lorenzana-Moreno *et al.* (2023) also noted an increase in CP when grape pomace and corn stover were bioprocessed with different strains of OYM. Crude protein plays a crucial role in sustaining the growth of rumen microbes, thereby promoting efficient fermentation and resulting in the optimal production of volatile fatty acids and microbial proteins (Abid *et al.*, 2023), thus maximizing ruminant output and production.

In contrast to CP, bioprocessing OP with OYM reduced crude fat content. This outcome was anticipated, as OYM can efficiently eliminate lipophilic compounds from lignocellulosic substrates, regardless of whether they are saponifiable or unsaponifiable (Rouches *et al.*, 2018). Consistent with our results, Brozzoli *et al.* (2010) observed a decrease in the crude fat content in stoned OP mixed with various conventional feedstuffs inoculated with either 10 g/L *Pleurotus ostreatus* or *P. pulmonarius* for 6 w at 28 °C. The reduction in the fat also points to the ability of OYM to utilize this component for energy. However, Abid *et al.* (2023) noted no changes in the crude fat content of grape pomace following treatment with either *P. cornucopiae* or *Ganoderma resinaceum* for 8 w at 25 °C. Ash content of OP increased upon bioprocessing with OYM. This could simply be an indicator of substrate decomposition, implying that as the OM fraction was utilized, the ash concentration increased. These findings are consistent with those of Lorenzana-Moreno *et al.* (2023), who noted that cultivating 17

strains of white rot fungi at a 5% spawn rate for 25 d on corn stover elevated the ash composition of the spent substrate.

Bioprocessing with oyster mushrooms is known to reduce fibre fractions in several agro-wastes (Nasehi *et al.*, 2017; Mhlongo *et al.*, 2021; Abid *et al.*, 2023). In this study, OYM effectively reduced NDF, ADF, and ADL content in OP, consistent with expectations. This indicates the breakdown of cell wall constituents in the substrates, facilitated by the extracellular enzymes of OYM (Kumla *et al.*, 2020). When grown on OP, OYM functions as a non-specific fungus capable of decomposing NSPs (Mhlongo *et al.*, 2021), the reason for the observed decrease in DM and OM in this study. Oyster mushrooms are equipped with two distinct, external enzymatic mechanisms: a hydrolytic system, responsible for generating hydrolyses to break down polysaccharides, and a unique oxidative and extracellular ligninolytic system designed for decomposing lignin and initiating the opening of phenyl rings (Andlar *et al.*, 2018). The current study revealed that there were no changes in hemicellulose and cellulose, which was unexpected, given the amorphous characteristics that typically make them susceptible to hydrolysis by the hemicellulolytic and cellulolytic complexes of OYM (Kumla *et al.*, 2020). Indeed, our results are at variance with earlier reports (Brozzoli *et al.*, 2010; Nie *et al.*, 2020; Abid *et al.*, 2023; Lorenzana-Moreno *et al.*, 2023) that indicated changes in hemicellulose and cellulose content in several agro-wastes bioprocessed with different strains of OYM.

Varying spawning rates led to positive quadratic responses for *in vitro* ruminal cumulative gas production across all the incubation periods measured (Table 2). Oyster mushroom spawning rates of 5.5 and 6.1% were determined to minimize cumulative gas production at 24 and 36 h, respectively.

**Table 2** *In vitro* cumulative gas production responses of olive pomace spent oyster mushroom substrate

| Incubation time (h) | Regression equation | $R^2$ | *P* value |
|---|---|---|---|
| 12 | $y = 0.003\ (\pm0.001)\ x^2 + 0.038\ (\pm0.042)\ x + 16.9\ (\pm0.435)$ | 0.836 | 0.0002 |
| 24 | $y = 0.003\ (\pm0.001)\ x^2 + 0.033\ (\pm0.042)\ x + 18.5\ (\pm0.441)$ | 0.837 | 0.0002 |
| 36 | $y = 0.004\ (\pm0.001)\ x^2 + 0.022\ (\pm0.049)\ x + 22.9\ (\pm0.512)$ | 0.848 | <0.0001 |
| 48 | $y = 0.005\ (\pm0.001)\ x^2 + 0.003\ (\pm0.053)\ x + 26.6\ (\pm0.560)$ | 0.852 | <0.0001 |
| 72 | $y = 0.006\ (\pm0.001)\ x^2 - 0.006\ (\pm0.059)\ x + 30.5\ (\pm0.620)$ | 0.862 | <0.0001 |
| 96 | $y = 0.007\ (\pm0.001)\ x^2 - 0.017\ (\pm0.064)\ x + 33.5\ (\pm0.667)$ | 0.868 | <0.0001 |

A varying OYM spawning rate effect was observed in *in vitro* ruminal cumulative gas production at all intervals measured (Table 3). To 36 h post-inoculation, OP0 and OP10 produced similar low cumulative gas production, followed by OP20 and OP30, whereas OP40 and OP50 produced the highest cumulative gas production. At 48–72 h post-inoculation, OP50 produced higher ($P$ <0.05) cumulative gas than OP0, which was similar to OP10 and OP20 ($P$ >0.05). At 96 h post-inoculation, OP50 produced the highest ($P$ <0.05) cumulative gas followed by OP40, OP30, and OP20, whereas OP0 and OP 10 produced the lowest cumulative gas.

**Table 3** Olive pomace spent oyster mushroom substrate *in vitro* cumulative gas production (mL/g OM)

| [2]Incubation time (h) | [1]Substrates | | | | | | [3]SEM | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | OP0 | OP10 | OP20 | OP30 | OP40 | OP50 | | *P-GLM* | *P-Linear* | *P-Quadratic* |
| 12 | 16.9[a] | 17.8[a] | 19.8[b] | 19.3[b] | 25.0[c] | 26.3[c] | 0.36 | <0.0001 | <0.0001 | 0.0002 |
| 24 | 18.4[a] | 19.2[a] | 21.2[b] | 20.8[b] | 26.6[c] | 27.9[d] | 0.37 | <0.0001 | <0.0001 | 0.0002 |
| 36 | 22.9[a] | 23.8[a] | 25.9[b] | 25.9[b] | 32.3[c] | 34.6[d] | 0.47 | <0.0001 | <0.0001 | <0.0001 |
| 48 | 26.5[a] | 27.2[ab] | 29.2[bc] | 29.8[c] | 36.6[d] | 39.3[e] | 0.54 | <0.0001 | <0.0001 | <0.0001 |
| 72 | 30.5[a] | 31.0[ab] | 33.3[bc] | 34.3[c] | 42.0[d] | 45.1[e] | 0.60 | <0.0001 | <0.0001 | <0.0001 |
| 96 | 33.6[a] | 33.9[a] | 36.6[b] | 37.7[b] | 46.1[c] | 49.6[d] | 0.65 | <0.0001 | <0.0001 | <0.0001 |

[abcd]In a row, different superscripts signify significant differences ($P$ <0.05) between spawning rate means
[1]Substrates: OP0 = uninoculated olive pomace, OP10–50 = olive pomace inoculated with 10–50% oyster mushroom spawn
[2]Incubation time: cumulative gas production at 12, 24, 36, 48, 72 and 96 h post-incubation
[3]SEM = standard error mean

Cumulative gas production increased with OYM bioprocessing throughout the 12–96 h incubation (Table 3). These findings align with those of Nasehi *et al.* (2017), who reported that bioprocessing of various agricultural residues with *P. florida* (5%) over 21 d at 25 °C increased *in vitro*

ruminal gas production. Several factors, including the nature and quantity of fibre and the potency of the rumen liquor used for incubation, can influence the gas volume during fermentation (Owens & Basalan, 2016). Generally, the gas volume is indicative of the extent and rate of degradability of carbohydrates, however, the type of carbohydrate substantially affects this parameter (Beuvink & Spoelstra, 1992; Chatellard *et al.*, 2016). Both NDF and ADF are known to exhibit a negative correlation with *in vitro* ruminal gas production (Nasehi *et al.*, 2017). Consequently, the reduced fibre fractions in OYM-cultivated OP led to increased gas production in the current study and as previously reported by Akinfemi *et al.* (2010). The decrease in NDF and ADF values tends to enhance microbial activity by creating more favourable ruminal growing conditions as the incubation period advances. Apart from higher fibre content, the lower gas production from untreated OP can also be ascribed to the higher crude fat content (Table 1). Higher fat content, as demonstrated by Bhatt *et al.* (2011), lowers microbial fermentation, resulting in low ruminal gas volumes. Whereas higher gas production does indicate enhanced microbial activity in the rumen due to more cellulose and hemicellulose being readily accessible for fermentation, this parameter does not always indicate improved nutritional value (Mhlongo *et al.*, 2021). This is because gases such as $CH_4$ and $CO_2$ reduce the dietary energy available to the animal (Renand *et al.*, 2019). As a result, increased gas production would be undesirable unless accompanied by increased OM degradability (Mhlongo *et al.*, 2021). Consequently, we employed PF, a ratio of gas production and organic matter degradability, to gain a clearer picture of the impact of OYM bioprocessing on the *in vitro* ruminal fermentation efficiency of OP.

Table 4 indicates that *a* and *c* linearly increased, whereas PF decreased linearly in response to increasing OYM spawning rates. Positive quadratic effects ($P$ <0.05) were noted for *b*, *Pgas*, *Egas*, and *iv*OMD in response to the varying OYM spawning levels. Gas production from the slowly degradable fraction (b) and ivOMD of spent OP substrate were minimized at 5.86 and 2.19% OYM spawning rate, respectively.

**Table 4** Polynomial regression of gas production kinetics, organic matter degradability, and partitioning factors in spent olive pomace substrate

| [1]Parameters | Regression equation | $R^2$ | *P* value |
|---|---|---|---|
| *A* | $y$ = 0.034 (±0.019) $x$ + 6.58 (±0.203) | 0.294 | <0.0001 |
| *B* | $y$ = 0.007 (±0.001) $x^2$ – 0.082 (±0.058) $x$ + 28.6 (±0.605) | 0.854 | 0.0001 |
| *C* | $y$ = 0.0001 (±0.0001) $x$ + 0.026 (±0.001) | 0.319 | <0.0001 |
| *Pgas* | $y$ = 0.007 (±0.001) $x^2$ – *0.048 (±0.063)* $x$ + 35.1 (±0.670) | 0.848 | <0.0001 |
| *Egas* | $y$ = 0.004 (±0.001) $x^2$ + 0.010 (±0.044) $x$ + 22.7 (±0.466) | 0.856 | <0.0001 |
| *ivOMD* | $y$ = 0.071 (±0.035) $x^2$ + 0.311 (±1.79) $x$ + 179 (±18.79) | 0.514 | 0.048 |
| PF | y = 0.194 (±0.009) – 0.001 (±0.001) $x$ | 0.229 | 0.0002 |

[1]Parameters: *a* = the immediately fermentable fraction, *b* = the slowly fermentable fraction, *c* = fermentation rate of fraction *b*, *Pgas* = potential gas production, *Ega*s = effective gas production, *iv*OMD= *in vitro* organic matter degradation, PF = partitioning factor at 96 h post incubation

All the measured *in vitro* gas production kinetics parameters showed variations ($P$ <0.05) in response to the increasing OYM spawning rates (Table 5). OP50 presented a higher *a* fraction than the OP0 but did not vary from OP20, OP30, and OP40. The OP0, OP10, and OP20 had the lowest fraction *b* followed by OP30 and OP40, whereas OP50 had the highest *b* fraction ($P$ <0.05). OP0, OP10, and OP30 had similar low *c* fractions, followed by OP20 and OP50, whereas OP40 had the highest value ($P$ <0.05). OP50 produced higher ($P$ <0.05) *Pgas* than OP0, which was in turn similar to OP10 and OP20. OP50 had the highest ($P$ <0.05) *Egas* followed by OP40, OP30, and OP20, whereas OP0 had the lowest with OP10. OP40 and OP50 had higher ($P$ <0.05) *iv*OMD than OP0, which was similar to OP10, OP20, and OP30. OP20 had a higher PF (0.02 mL/mg OM) than OP30, OP40, and OP50, but similar values to OP0 and OP10.

Increased gas production from the readily fermentable OM (*a*) in bioprocessed OP shows the potential of this strategy to improve agricultural waste. Bioconversion of OP into a more readily-fermentable substrate implies that our study contributes to the sustainable utilization of agricultural by-products in ruminant nutrition. An enhanced *a* fraction in OYM-bioconverted substrates has been demonstrated previously (Nasehi *et al.*, 2017; Mhlongo *et al.*, 2021). Gas production from fraction *b* of bioprocessed OP had a positive quadratic effect, consistent with the findings of Mhlongo *et al.* (2021). Similarly, Akinfemi *et al.* (2010) and Nasehi *et al.* (2017) also reported an improved *b* fraction for agro-

residues following OYM cultivation, which can be attributed to the degradation of lignin, allowing rumen microbes to efficiently digest cellulose and hemicellulose in cultivated substrates.

**Table 5** Gas production kinetics, organic matter degradability (g/kg DM), and partitioning factors (mL/mg OM, unless otherwise stated) in spent olive pomace substrate

| | [1]Substrates | | | | | | | Significance | | |
|---|---|---|---|---|---|---|---|---|---|---|
| [2]Parameters | OP0 | OP10 | OP20 | OP30 | OP40 | OP50 | [3]SEM | *P-GLM* | *P-Linear* | *P-Quadratic* |
| *a* | 6.49$^{a}$ | 6.94$^{ab}$ | 7.46$^{bc}$ | 7.29$^{abc}$ | 7.51$^{bc}$ | 8.16$^{c}$ | 0.22 | <.0001 | <0.0001 | 0.702 |
| *b* | 28.9$^{a}$ | 28.1$^{a}$ | 29.6$^{a}$ | 32.2$^{b}$ | 38.5$^{c}$ | 41.9$^{d}$ | 0.64 | <.0001 | <0.0001 | <0.0001 |
| *c* | 0.025$^{a}$ | 0.028$^{abc}$ | 0.030$^{bcd}$ | 0.026$^{ab}$ | 0.033$^{d}$ | 0.031$^{cd}$ | 0.001 | <0.0001 | <0.0001 | 0.850 |
| *Pgas* | 35.4$^{a}$ | 35.1$^{a}$ | 37.1$^{ab}$ | 39.4$^{b}$ | 46.0$^{c}$ | 50.0$^{d}$ | 0.72 | <0.0001 | <0.0001 | <0.0001 |
| *Egas* | 22.6$^{a}$ | 23.2$^{a}$ | 25.1$^{b}$ | 25.5$^{b}$ | 31.3$^{c}$ | 33.5$^{d}$ | 0.44 | <0.0001 | <0.0001 | <0.0001 |
| *iv*OMD | 182$^{a}$ | 190$^{a}$ | 194$^{a}$ | 262$^{ab}$ | 316$^{bc}$ | 361$^{c}$ | 21.0 | <0.0001 | <0.0001 | 0.048 |
| PF | 0.19$^{ab}$ | 0.18$^{ab}$ | 0.20$^{b}$ | 0.15$^{a}$ | 0.15$^{a}$ | 0.15$^{a}$ | 0.010 | 0.002 | 0.0002 | 0.728 |

[abcde]In a row, different superscripts signify significant differences ($p < 0.05$) between spawning rate means

[1]Substrates: OP0 = uninoculated olive pomace, OP10–50 = olive pomace inoculated with 10–50% oyster mushroom spawn

[2]Parameters: *a* = the immediately fermentable fraction, *b* = the slowly fermentable fraction, *c* = fermentation rate of fraction *b*, *Pgas* = potential gas production, *Ega*s = effective gas production, *iv*OMD= *in vitro* organic matter degradation, PF = partitioning factor at 96 h post incubation

[3]SEM = standard error of the mean

The fermentation rate of fraction *b* (*c*) can be a crucial measure of feed availability and the production of microbial proteins (Owens & Basalan, 2016). Oyster mushrooms cultivated on OP affected fraction *c*, with OP40 substrate being fermented the fastest, which was anticipated since an increase in OYM spawning rate also enhanced the degradable portion of fractions *a* and *b*. Our findings indicated that increasing the OYM spawning rate enhanced the *Pgas*, *Egas*, and *iv*OMD, which can be attributed to reductions in NDF, ADF, and ADL and improved CP content in OYM-treated OP. These findings concur with previous reports (Brozzoli *et al.*, 2010; Nasehi *et al.*, 2017; Mhlongo *et al.*, 2021). The improved gas production and OM degradation suggest an enhanced release of nutrients such as sugars, amino acids, and fatty acids (Rouches *et al.*, 2016; Uwineza *et al.*, 2023) from bioprocessed OP. These nutrients support microbial growth and metabolic activities. Based on the formula for PF used in this study, lower values suggest higher ruminal fermentation efficiency since these imply that lower volumes of gas were produced for every gram of OM degraded. In the current study, we observed a linear decrease in the PF values at 96 h in response to increasing OYM spawning rates. This finding was expected given that OYM improved *iv*OMD by reducing fibre and increasing the crude protein content of OP substrates. This *iv*OMD enhancement was not accompanied by a proportional increase in gas production resulting in lower PF values and higher ruminal fermentation efficiency where OM degradation produced microbial biomass rather than methane and carbon dioxide.

## Conclusion

The cultivation of oyster mushrooms on olive pomace reduced the fibre fractions and crude fat while enhancing crude protein, which resulted in improved *in vitro* ruminal organic matter degradability and fermentation efficiency of the spent substrates. In conclusion, the enhanced nutritional value highlights the beneficial effect of oyster mushroom cultivation on olive pomace. These preliminary findings suggest that oyster mushrooms could be an effective tool for the value-adding to olive pomace for ruminants. While the bioprocessing of olive pomace for ruminant feed using oyster mushrooms is effective, its success depends strongly on managing the energy requirements of substrate sterilization, possibly through low-energy, alternative sterilization techniques, as well as process optimization to eliminate this step. The sustainability and economic efficiency of this strategy can be enhanced by accounting for broader benefits in waste reduction, resource re-use, increased animal productivity, reduction in methane emissions, and value addition.

**Conflict of interest**

The authors declare no conflict of interest.

**Author Contributions**

CMM, SIK, NNS, CFE, and VM conceptualised the study. SIK and NNS collected the data. CMM conducted the statistical analysis. SIK, NS, and CFE wrote the initial draft. CMM and VM reviewed the initial and final draft. All authors read and approved the final version of the manuscript.

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