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EFFECTS OF SOLID-STATE FERMENTATION WITH WHITE-ROT FUNGI ON THE NUTRITIVE VALUE OF GRAPE STALKS AS RABBIT FEED

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Resumo: Este estudo teve como objetivo estudar o efeito da inoculação de fungos da podridão branca no valor nutritivo do engaço de uva (co-produto da indústria vinícola), nomeadamente na composição química e na digestibilidade *in vitro* da matéria orgânica (IVOMD) em coelhos. Foram utilizadas três estirpes de fungos: *Lentinula edodes* (UF21403), *Pleurotus eryngii* (UF21402) e *Pleurotus citrinopileatus* (UF21401). O substrato foi inoculado em quadruplicado com os diferentes inóculos fúngicos por 4 períodos (0, 28, 35 e 42 dias) em condições controladas de temperatura e humidade. Os resultados indicaram que o tratamento com *P. citrinopileatus* aos 42 dias promoveu um maior aumento no teor em proteína bruta (74,8%) em relação ao controlo. No entanto, a maior redução nos teores dos componentes da parede celular foi obtida pelo tratamento com *L. edodes* aos 42 dias, com redução da fibra do detergente neutro (NDF), fibra do detergente ácido (ADF) e de lenhina de 10,6%, 16,8% e 45,1%, respetivamente, assim como o aumento da DIVMO em cerca de 19,6%. Os dados indicam que o fungo *L. edodes* possui maior capacidade para a valorização nutricional do engaço de uva para a alimentação de animais herbívoros.

Key-words: biological treatment, co-products, *in vitro* digestibility

Introduction

In recent years there is a trend towards a zero waste policy, where the efficient utilization of agricultural co-products is a vital concern. The insufficient storage and improper disposal of bio-waste may generate serious pollution problems and a significant loss of biomass. Like many agricultural residues, grape stalks are not attractive as an animal feed due to its high lignin contents and other anti-nutritional factors, with consequent negative effects on digestibility. For that reason, a possible solution for the reduction of lignin may be the biological treatment with white-rot fungi. White-rot fungi have been studied for the delignification of wood and other lignocellulosic materials due to their potential to produce ligninolytic enzymes that decrease the content in lignin and subsequently increase the digestibility of substrates. The objective of this study was to evaluate white-rot fungi as a possible biological treatment to enhance the nutritive value of grape stalks. Therefore, we intended to evaluate the effects of the solid-state fermentation on chemical composition and *in vitro* organic matter digestibility (IVOMD), in order to analyse the potential of grape stalks as rabbit feed.

Material and methods

The grape stalks samples were collected from region of Trás-os-Montes and Alto Douro, Portugal, in 2016. All the samples were immediately dried at 40°C and then milled to 1 cm for fungal colonization process. Three strains of fungi were used: *Lentinula edodes* (UF21403), *Pleurotus citrinopileatus* (UF21401) and *Pleurotus eryngii* (UF21402). Incubation was conducted in 500mL Erlenmeyer flasks containing 50g humidified substrate. Flasks were autoclaved at 121°C for 30 min, cooled, and inoculated with about 2g of inoculum spawn. After being thoroughly mixed, the incubations (quadruplicate per treatment) were conducted for 0, 28, 35 and 42 days at 28°C and 90% of relative humidity. After each incubation time, samples were dried to constant weight in an air-forced oven at 40°C and ground over a 1 mm screen for

subsequent analysis. Samples were analysed for dry matter (DM), organic matter (OM), ash and protein according to the procedures recommended by AOAC (1990). Determination of the cell wall constituents - neutral detergent fibre (NDF), acid detergent fibre (ADF) and lignin - was performed based on the methods proposed by Robertson and van Soest (1981). *In vitro* organic matter digestibility (IVOMD) for rabbits was calculated following the procedures described by Ramos et al. (1992).

Statistical analysis was carried out in the statistical program SAS (2009). The effect of the substrate (grape stalk), the fungi used *(L. edodes, P. citrinopileatus* and *P. eryngii*) and incubation time (0, 28, 35 and 42 days) and their interaction on the chemical composition and IVOMD was analysed by a two-way analysis of variance (ANOVA). Comparison of the means was performed using the Tukey test at a significance level of 5%.

Results and discussion

According to Table 1, it was observed an effect of the fungi on the chemical composition of the treated grape stalk, where all fungi decreased DM and OM, and increased ash and CP content *(P* <0.001). The data indicated that treatment with *P. citrinopileatus* resulted in the greatest increase in CP content, about 74.8% compared to control. This effect was also observed by Tuyen et al. (2012) and can be partially explained by a proportional reduction of other chemical compounds, such as the lignin content. Solid fermentation promoted distinct changes in the cell wall. In fact, *L. edodes* at 42 days resulted in a significant decrease in NDF (10.6%), ADF (16.8%) and lignin (45.1%) compared to control. In contrast, incubation with *P. eryngii* resulted in an increase in NDF and ADF content and no effect on the lignin contents.

Table 1- Chemical composition (%DM) and *in vitro* organic matter digestibility (IVOMD) for rabbits (%) of the treated substrates and controls with the different fungi during the different incubation times

	Chemical composition							
	% DM							
	DM	OM	Ash	CP	NDF	ADF	Lignin	IVOMD
Treatment								
Control	29,9^{a}	94,4^{a}	5,6^{d}	4,8^{d}	67,8^{b}	62,0^{a}	33,5^{a}	32,5^{b}
L. edodes	24,2^{c}	92,3^{c}	7,7^{b}	7,7^{b}	61,9^{c}	52,9^{c}	22,1^{c}	35,5^{a}
P. citrinopileatus	22,2^{d}	91,6^{d}	8,4^{a}	8,4^{a}	67,7^{b}	59,0^{b}	28,7^{b}	29,6^{c}
P. eryngii	27,4^{b}	93,5^{b}	6,5^{c}	6,4^{c}	70,8^{a}	63,9^{a}	32,5^{a}	30,4^{c}
Time								
0 days	25,8^{ab}	94,3^{a}	5,7^{b}	5,8^{b}	65,7^{b}	59,0^{ab}	31,2^{a}	33,1^{a}
28 days	25,1^{b}	92,5^{b}	7,5^{a}	7,1^{a}	68,7^{a}	60,7^{a}	29,8^{ab}	30,7^{b}
35 days	26,2^{ab}	92,5^{b}	7,5^{a}	7,1^{a}	67,3^{b}	59,7^{ab}	28,8^{b}	32,0^{a}
42 days	26,6^{a}	92,5^{b}	7,5^{a}	7,4^{a}	66,4^{b}	58,3^{b}	27,0^{c}	32,2^{a}
SEM	0,57	0,15	0,15	0,17	0,48	0,69	0,56	0,41
Effect								
Treatment	***	***	***	***	***	***	***	***
Time	NS	***	***	***	***	*	***	***
Treatment x Time	***	***	***	***	***	***	***	***

DM, dry matter; OM, organic matter; CP, crude protein; NDF, neutral detergent fibre; ADF, acid detergent fibre; IVOMD, *in vitro* organic matter digestibility for rabbits; SEM, standard error of mean. Values with different superscripts within row are significantly different (P <0.05) according to the Tukey test; * P <0.05; ** P <0.01; *** P <0.001; NS, not significant.

Regarding IVOMD the results indicate that treatment with *L. edodes* at 42 days resulted in an increase of about 19.6% compared to control. These results are consistent with Andrade et al. (2017), which indicate a 31% increase in IVOMD when inoculating cowpea straw with *P. citrinopileatus*. These differences can be explained by specific modifications in the cell wall structure promoted by white-rot fungi during the incubation process, depending on the fungal strains used, the incubation process and the chemical characteristics of the substrates.

Conclusion

Incubation of grape stalks with *L. edodes* promoted a significant reduction in the lignin content and an appreciable increase in IVOMD. The results indicate that the incubation period affected the nutritive value of the grape stalks, indicating that the use of longer incubation times may result in a greater decrease in lignin content.

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