

DIENE FRANCE DE SOUZA

**PRODUÇÃO DE COGUMELOS DE *Pleurotus ostreatus* ENRIQUECIDOS COM
SELÊNIO E ALIMENTAÇÃO DE SUÍNOS PARA ESTUDOS DE
BIODISPONIBILIDADE DO MINERAL**

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Ciência e Tecnologia de Alimentos, para obtenção do título de *Doctor Scientiae*.

Orientadora: Monique Renon Eller

Coorientadores: Gabriel Cipriano Rocha
Maria Catarina Megumi Kasuya
Marliane de Cássia Soares da Silva

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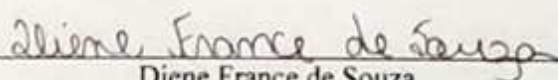
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Assentimento:


Diene France de Souza
Autora


Monique Renon Eller
Orientadora

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RESUMO

SOUZA, Diene France de, D.Sc., Universidade Federal de Viçosa, novembro de 2021. **Produção de cogumelos de *Pleurotus ostreatus* enriquecidos com selênio e alimentação de suínos para estudos de biodisponibilidade do mineral.** Orientadora: Monique Renon Eller. Coorientadores: Gabriel Cipriano Rocha, Maria Catarina Megumi Kasuya e Marliane de Cássia Soares da Silva.

Cogumelos incorporam, a partir do substrato, diversos minerais essenciais à dieta humana, como o selênio. Este estudo objetivou avaliar a influência da adição do selênio no substrato de cultivo sobre o rendimento, composição e atividade antioxidante de cogumelos de *Pleurotus ostreatus* e estudar a biodisponibilidade desse mineral em leitões alimentados com rações contendo cogumelos enriquecidos. Os resultados foram apresentados em três capítulos. No primeiro, uma revisão bibliográfica sobre o tema permitiu concluir que a adição do selênio em baixas concentrações (até 17,5 mg kg⁻¹) ao substrato de cultivo de cogumelos aumenta sua eficiência biológica, teor de proteínas e de compostos fenólicos. Além disso, esses cogumelos selenizados são antioxidantes e antitumorais. No capítulo 2 é descrito o uso de selenito de sódio (25 mg kg⁻¹ de selênio) para enriquecer cogumelos de *P. ostreatus* cultivados em substratos compostos por cinco proporções de bagaços de cana-de-açúcar e de malte de cevada, imersos em solução alcalina (Ca(OH)₂) a 2 % (m v⁻¹), visando avaliar a influência do selênio e do substrato no rendimento e na composição dos cogumelos. Somente o bagaço de cana-de-açúcar puro ou em combinação com 25 % de bagaço de malte de cevada levaram ao desenvolvimento de cogumelos, com uma eficiência biológica de 50,89 e 37,80 g 100 g⁻¹, respectivamente. Nos demais substratos larvas desenvolveram-se. Os cogumelos selenizados derivados daqueles substratos continham 179,07 e 127,03 µg g⁻¹ de selênio, respectivamente. Assim, o bagaço de cana-de-açúcar foi considerado o melhor substrato para cultivo de cogumelos selenizados, nas condições testadas. No capítulo 3 descrevemos os experimentos realizados com o objetivo de avaliar se existe diferença sobre o desempenho, biodisponibilidade plasmática do mineral e atividade antioxidante plasmática e hepática de leitões desmamados alimentados com ração contendo cogumelos selenizados ou não, em comparação aos parâmetros de animais alimentados com rações contendo leveduras selenizadas (produto padrão). Para isso, 64 animais foram divididos em quatro grupos, que receberam dietas à base de farelos de milho e de soja (controle - G1), acrescida de cogumelos (G2), cogumelos selenizados (G3) ou leveduras selenizadas (G4). A dieta foi fornecida *ad*

libitum por 21 dias. Independentemente da dieta, o teor de selênio (19,42 a 20,88 $\mu\text{g L}^{-1}$) e a atividade da glutationa-peroxidase (Gpx) (75,74 a 79,09 U mL^{-1}) foram similares, indicando que nenhuma dieta prejudicou o equilíbrio plasmático. No fígado, a atividade das enzimas Gpx (23,17 a 26,51 $\text{U mg proteína}^{-1}$), catalase (23,27 a 29,28 $\text{U mg proteína}^{-1}$) e superóxido dismutase (1,54 a 1,80 $\text{U mg proteína}^{-1}$) foi maior nos leitões alimentados com rações contendo cogumelos (selenizados ou não) e leveduras selenizadas em comparação à dieta controle, sendo que nesse caso, a atividade das enzimas Gpx, catalase e superóxido dismutase foi de 14,55, 16,18 e 1,06 $\text{U mg proteína}^{-1}$, respectivamente. Concluimos que o consumo dos cogumelos selenizados ou não foi seguro e levou a desempenho e atividade antioxidante semelhantes à alimentação com o padrão comercial atual (levedura selenizada). Isso evidencia o potencial de uso de cogumelos como alimento e como veículo de minerais para a suplementação da alimentação animal e humana.

Palavras-chave: Selenito de sódio. Produção de cogumelos. Resíduos agroindustriais. Leitões.

ABSTRACT

SOUZA, Diene France de, D.Sc., Universidade Federal de Viçosa, November, 2021. **Production of selenium-enriched *Pleurotus ostreatus* mushroom and swine feed for study of selenium bioavailability.** Adviser: Monique Renon Eller. Co-advisers: Gabriel Cipriano Rocha, Maria Catarina Megumi Kasuya and Marliane de Cássia Soares da Silva.

Mushrooms incorporate, from the substrate, several minerals essential to the human diet, such as selenium. This study aimed to evaluate the influence of selenium addition to the culture substrate on the yield, composition and antioxidant activity of *Pleurotus ostreatus* mushrooms and to study the bioavailability of this mineral in piglets fed rations containing enriched mushrooms. The results were presented in three chapters. In the first, a literature review on the subject concluded that the addition of selenium at low concentrations (up to 17.5 mg kg^{-1}) to the mushroom growing substrate increases its biological efficiency, protein content and phenolic compounds. In addition, these selenized mushrooms are antioxidant and antitumor. Chapter 2 describes the use of sodium selenite (25 mg kg^{-1} of selenium) to enrich *P. ostreatus* mushrooms grown on substrates composed of five proportions of sugarcane bagasse and brewer's spent grain, immersed in 2 % (m v^{-1}) alkaline solution (Ca(OH)_2), to evaluate the influence of selenium and substrate on mushroom yield and composition. Only sugarcane bagasse alone or in combination with 25 % of brewer's spent grain led to mushroom development with a biological efficiency of 50.89 and $37.80 \text{ g } 100 \text{ g}^{-1}$, respectively. On the other substrates larvae developed. The selenized mushrooms derived from those substrates contained 179.07 and $127.03 \text{ } \mu\text{g g}^{-1}$ of selenium, respectively. Thus, sugarcane bagasse was considered the best substrate for growing selenized mushrooms under the conditions tested. In Chapter 3 we describe the experiments carried out to evaluate whether there is difference in performance, plasma bioavailability of the mineral and plasma and liver antioxidant activity in weaned piglets fed rations containing selenized or non-selenized mushrooms compared to animals fed rations containing selenized-yeast (standard product). For this purpose, 64 animals were divided into four groups, which received diets based on corn and soybean meal (control - G1), plus mushrooms (G2), selenized mushrooms (G3) or selenized-yeast (G4). The diet was fed ad libitum for 21 days. Regardless of diet, selenium content (19.42 to $20.88 \text{ } \mu\text{g L}^{-1}$) and glutathione peroxidase (Gpx) activity (75.74 to 79.09 U mL^{-1}) were similar, indicating that no diet impaired plasma balance. In the liver, the activity of the enzymes Gpx (23.17 to $26.51 \text{ U mg protein}^{-1}$), catalase (23.27 to $29.28 \text{ U mg protein}^{-1}$), and superoxide dismutase

(1.54 to 1, 80 U mg protein⁻¹) was higher in piglets fed feeds containing mushrooms (selenized or not) and selenized-yeast compared to the control diet, in which case the activity of the enzymes Gpx, catalase and superoxide dismutase was 14.55, 16.18 and 1.06 U mg protein⁻¹, respectively. We conclude that consumption of the selenized or non-selenized mushrooms was safe and led to similar performance and antioxidant activity to feeding the current commercial standard (selenized-yeast). This highlights the potential for using mushrooms as a food and as a carrier of minerals for supplementation of animal and human nutrition.

Keywords: Sodium selenite. Mushrooms production. By-products. Piglets.

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INTRODUÇÃO GERAL

Os cogumelos são apreciados por muitas culturas, podendo ser preparados cozidos, assados, grelhados ou mesmo *in natura*, na forma de saladas. Estima-se que haja em torno de 45 mil espécies de cogumelos no mundo (SOTO, 2019), das quais duas mil são consideradas comestíveis, mas somente quatro se destacam comercialmente: *Agaricus bisporus* (Champignon), *Lentinula edodes* (Shiitake), *Pleurotus ostreatus* (cogumelo ostra) e *Volvariella volvaceae* (cogumelo palha) (CHANG & MILES, 2004).

Cogumelos comestíveis são constituídos (em base seca) principalmente por carboidratos (45-80 %) e proteínas (18-35 %), enquanto o teor de lipídios geralmente é menor que 4 % (CHANG & MILES, 2004). Eles também possuem vitaminas e compostos antioxidantes, como os fenólicos (ANWAR, HUSSAIN & MUSTAFA, 2018), além de minerais, com predominância de potássio, fósforo, sódio, cálcio e magnésio, que correspondem a 56-70 % do total de cinzas (CHANG & MILES, 2004). Os cogumelos ainda incorporam outros minerais, como o selênio, a partir do substrato de cultivo (DA SILVA et al., 2012).

Para o cultivo de cogumelos selenizados, resíduos agroindustriais lignocelulósicos, como os bagaços de malte de cevada (WANG, SAKODA & SUZUKI, 2001) e de cana-de-açúcar (NUNES et al., 2012), dentre outros, podem ser usados, o que poderia viabilizar um alimento de alto valor agregado com baixo custo. Algumas das características que devem nortear a escolha do substrato são a disponibilidade, o rendimento de produção e sua inocuidade. Por exemplo, substratos que contenham excesso de determinados minerais, como cádmio e arsênio, podem originar cogumelos tóxicos, já que estes absorvem os nutrientes para seu desenvolvimento a partir do substrato (OYETAYO & ARIYO, 2013). A depender do

substrato, a composição e, conseqüentemente, o valor nutricional dos cogumelos podem variar (OYETAYO & ARIYO, 2013).

Substratos como cascas de café, sabugo de milho e bagaço de cana-de-açúcar já foram testados para a produção de cogumelos de *P. ostreatus* (DA SILVA et al., 2012; NUNES et al., 2017), incluindo cogumelos selenizados. Porém, a maioria dos trabalhos na área utilizam a esterilização para a desinfestação do substrato (DA SILVA et al., 2012), o que tornaria o processo de produção desses alimentos mais oneroso. Por isso, é necessário o desenvolvimento de tratamentos alternativos para a desinfestação, como a imersão em solução alcalina (NUNES et al., 2017) e é preciso também que substratos abundantes no Brasil sejam testados, como o bagaço de malte de cevada.

Nesse contexto, a produção do cogumelo ostra (*P. ostreatus*) apresenta como vantagens a não exigência de compostagem prévia do substrato, diferentemente do que ocorre no cultivo de Champignon (PHILIPPOUSSIS, 2009), e o fato de seu ciclo de cultivo ser de aproximadamente 30 dias, sendo menor que o ciclo de cultivo do Shiitake, o qual pode ser superior a 6 meses (PICCININ, 2000). Além disso, o *P. ostreatus* converte um alto percentual do substrato em corpo de frutificação, mesmo na presença de minerais para enriquecimento, como o selênio (DA SILVA et al., 2012).

O selênio é um elemento cuja ingestão em excesso pode causar fadiga, fragilidade de unhas, paralisia e até mesmo a morte, porém em baixas concentrações é essencial aos organismos (NUTTALL, 2006), sendo que sua deficiência na alimentação humana pode causar disfunções da tireóide, agravar infecções, elevar a incidência de câncer, aumentar o risco de doenças cardiovasculares e fazer com que haja complicações do diabetes *mellitus* (HATFIELD, 2001). Além disso, o selênio auxilia na regeneração molecular, na reprodução e no desenvolvimento embrionário (COMINETTI & COZZOLINO, 2009). O baixo consumo de selênio pode causar a doença de Keshan, caracterizada por insuficiência cardíaca de níveis

diversos e a doença de Kashin-Beck, que pode ocorrer na pré e na adolescência, caracterizada pela osteoartrite endêmica (HATFIELD, 2001). A recomendação de ingestão e o limite máximo tolerável são de 55 µg e 400 µg por dia, respectivamente (IOM, 2001).

No Brasil, o consumo de selênio em excesso não é um problema comum, e sim sua baixa ingestão, pois a concentração de selênio em solos de algumas regiões é extremamente baixa ou mesmo nula (MORAES et al., 2009). Por isso, pode haver deficiência desse mineral na alimentação do brasileiro. Dessa forma, a ingestão de alimentos naturalmente ricos em selênio, como brócolis, nabo, rabanetes, alho, cebola e castanha-do-Pará, pode não ser suficiente, e a suplementação é uma alternativa para suprir essa carência (PRAUCHNER, 2014). A biofortificação de alimentos com selênio pode ser feita pela adição desse mineral no meio no qual os alimentos, como cogumelos, serão cultivados (DA SILVA et al., 2012). Assim, quando os cogumelos são enriquecidos, poderão fornecer o selênio complementar à dieta, além de seus benefícios nutricionais (DA SILVA et al., 2012; SOUZA et al., 2021).

Quando ingerido, o selênio é metabolizado principalmente no fígado e nos rins e é excretado principalmente nas fezes e na urina (SUZUKI et al., 2010). Existem estudos que relatam os efeitos do consumo de cogumelos selenizados por animais, normalmente ratos (DA SILVA et al., 2010; MASEKO et al., 2014). Entre algumas das principais alterações observadas destacam-se a elevação da atividade da enzima glutathione-peroxidase (MASEKO et al., 2014) e da biodisponibilidade do mineral para o organismo (DA SILVA et al., 2010). Porém, é essencial a utilização de modelos animais que tenham maior similaridade ao organismo humano, como os suínos. As características do DNA e dos órgãos desses animais, como estômago, intestino, fígado, rins e coração, assemelham-se às humanas, por isso, os suínos são tradicionalmente utilizados para experimentação visando à extrapolação de resultados para entendimento dos efeitos de certos componentes para tratamentos de condições que afetam os seres humanos, como obesidade e diabetes (AJUWON, 2017).

Os resultados derivados do presente estudo estão descritos em três capítulos. No capítulo 1 foram compiladas informações relevantes sobre os efeitos do uso do selênio no meio de cultivo de cogumelos sobre o rendimento, composição e atividade antioxidante e os efeitos do uso desses alimentos ou de seus derivados sobre atividade antioxidante e antitumoral em animais. No capítulo 2 são descritos os resultados do experimento cujo objetivo foi verificar o efeito do uso do selênio sobre o rendimento, composição e atividade antioxidante de cogumelos de *P. ostreatus*, produzidos em substratos compostos por bagaços de cana-de-açúcar e de malte de cevada, imersos em solução alcalina. Por fim, no capítulo 3, são descritos os efeitos da ingestão de cogumelos de *P. ostreatus* enriquecidos ou não com selênio sobre a atividade antioxidante e a biodisponibilidade do mineral em modelo porcino.

REFERÊNCIAS

- AJUWON, K. M. The pig model for the study of obesity and associated metabolic diseases. In: SCANIS, C. G.; HILL, R. A. (Eds). **Biology of domestic animals**. 370 p. Boca Raton: CRC Press, 2017. Cap. 2, p. 18-47.
- ANWAR, H.; HUSSAIN, G.; MUSTAFA, I. Antioxidants from natural sources. In: SHALABY, E.; AZZAM, G. M. (Eds). **Antioxidants in foods and its applications**. 178 p. London: IntechOpen, 2018. Cap. 1, p. 3-28.
- CHANG, S. T.; MILES P. G. **Mushrooms: cultivation, nutritional value medicinal effect and enviromental impact**. Boca Raton: CRC Press. 2004. 480 p.
- COMINETTI, C.; COZZOLINO, S. M. F. Selênio. **Funções plenamente reconhecidas de nutrientes**. São Paulo, 2009, v. 08. p. 1-24.
- DA SILVA, M. C. S.; NAOZUKA, J.; LUZ, J. M. R.; ASSUNÇÃO, L. S.; OLIVEIRA, P. V.; VANETTI, M. C. D.; BAZZOLLI, D. M. S.; KASUYA, M. C. M. Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. **Food chemistry**, v. 131, n. 2, p. 558-563 mar. 2012.
- DA SILVA, M. C. S.; NAOZUKA, J.; OLIVEIRA, P. V.; VANETTI, M. C. D.; BAZZOLLI, D. M. S.; COSTA, N. M. B.; KASUYA, M. C. M. *In vivo* bioavailability of selenium in enriched *Pleurotus ostreatus* mushrooms. **Metallomics**, v. 2, p. 162-166, jan. 2010.
- HATFIELD, D. L. **Selenium: Its molecular biology and role in human health**. 1 ed. Boston: Kluwer Academic Publishers, 2001. 352 p.
- INSTITUTE OF MEDICINE. Food and nutrition board. 2001. **Dietary Reference Intakes**. National Academic Press: Washington D. C.
- MASEKO, T.; HOWELL, K.; DUNSHEA, F. R.; NG, K. Selenium-enriched *Agaricus bisporus* increases expression and activity of glutathione peroxidase-1 and expression of glutathione peroxidase-2 in rat colon. **Food chemistry**, 146, 327–333, 2014.
- MORAES, M. F.; WELCH, R. M.; NUTTI, M. R.; CARVALHO, J. L. V.; WATANABE, E. Evidences of selenium deficiency in Brazil: from soil to human nutrition. In:

- BANUELOS, G. R.; LIN, Z. G.; YIN, X. B. (Eds). First International Conference on Selenium in the Environment and Human Health, Suzhou. **Selenium: deficiency, toxicity and biofortification for human health**. 116p. Hefei: University of Science and Technology of China Press, 2009. p.73-74.
- NUNES, M. D.; DA SILVA, M. C. S.; SCHRAM, J. G. S. et al. *Pleurotus ostreatus*, mushrooms production using quick and cheap methods and the challenges to the use of coffee husk as substrate. **African Journal of Microbiology Research**, v. 11, p. 1252–1258, 2017.
- NUNES, M. D.; LUZ, J. M. R.; PAES, S. A.; RIBEIRO, J. J. O.; DA SILVA, M. C. S.; KASUYA, M. C. S. Nitrogen supplementation on the productivity and the chemical composition of oyster mushroom. **Journal of food research**, v.1, n. 2, p. 113-119, 2012.
- NUTTALL, K. L. Evaluating selenium poisoning. *Animals of clinical and laboratory sciences*, v. 36, p.409-420, 2006.
- OYETAYO, V. O.; ARIYO, O. O. Micro and macronutrient properties of *Pleurotus ostreatus* (Jacq:Fries) cultivated on different wood substrates. **Jordan journal of biological sciences**, v. 6, n. 3, p. 223–226, 2013.
- PHILIPPOUSSIS, A. Production of mushrooms using agro-industrial residues as substrates. In: **Biotechnology for Agro-Industrial Residues Utilisation: Utilisation of Agro-Residues**; Singh nee' Nigam, P., Pandey, A., Eds. Springer: Dordrecht, The Netherlands, 2009. p. 163–196.
- PICCININ, E. **Cultivo do cogumelo Shiitake (*Lentinula edodes*) em toras de eucalipto: Teoria e Prática**. Série Produtor Rural, edição especial, Piracicaba, 2000. 48 p.
- PRAUCHNER, C. A. **A importância do selênio para a agropecuária e saúde humana**. Editora UFSM: Santa Maria, 2014. 376 p.
- SOTO, B. Fermentation in food processing: microbiology and biotechnology. In: SOTO, B. (Ed.). **Fermentation processes**. ED Tech Press, 2019. Cap. 7, p. 173–248.

SOUZA, D. F.; DA SILVA, M. C. S.; ALVES, M. P. et al. By-products as substrates for production of selenium-enriched *Pleurotus ostreatus* mushrooms. **Waste and Biomass Valorization**, 2021.

SUZUKI, Y.; HASHIURA, Y.; MATSUMURA, K.; MATSUKAWA, T.; SHINOHARA, A.; FURUTA, N. Dynamic pathways of selenium metabolism and excretion in mice under different selenium nutritional stresses. **Metallomics**, v. 2, p. 126-132, 2010.

WANG, D.; SAKODA, A.; SUZUKI, M. Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. **Bioresource technology**, v. 78, n. 3, p. 293-300, jul. 2001.

CAPÍTULO 1: Nutritional and functional aspects of selenium-enriched mushrooms

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Nutritional and functional aspects of selenium-enriched mushrooms

Diene France de Souza¹, Marliane de Cássia Soares da Silva², Tainara Camila de Souza³, Gabriel Cipriano Rocha⁴, Maria Catarina Megumi Kasuya², Monique Renon Eller^{1,*}

1 Department of Food Technology, Universidade Federal de Viçosa (UFV), Viçosa, MG, 36570-900, Brazil.

2 Department of Microbiology, Universidade Federal de Viçosa (UFV), Viçosa, MG, 36570-900, Brazil.

3 Department of Chemistry, Universidade Federal de Viçosa (UFV), Viçosa, MG, 36570-900, Brazil.

4 Department of Animal Science, Universidade Federal de Viçosa (UFV), Viçosa, MG, 36570-900, Brazil.

* Corresponding author.

E-mail address: monique.eller@ufv.br (M.R. Eller)

Abstract

Mushrooms absorb minerals from substrates in which they are cultivated, and thus they could be used as vectors for minerals that are deficient in human or animal diets, such as selenium. Selenium deficiency aggravates cardiovascular diseases, diabetes *mellitus*, and intestinal cancer. The selenium from selenized mushrooms is bioaccessible and bioavailability. Selenized mushrooms and their derivatives, such as selenopolysaccharides, present antioxidant and antitumor activity. These properties have been demonstrated *in vitro* and confirmed *in vivo*. In addition, the consumption of these foods reduces the levels of cholesterol and glucose in the blood. On other hand, growing mushrooms in a substrate enriched with selenium may alter the biological efficiency and their chemical composition. In this article, the enrichment of mushrooms with selenium is reviewed, focusing on the use of edible mushrooms as an alternative to supplying the deficiency of this mineral in diets.

Keywords: sodium selenite, selenium enrichment, antioxidant activity, mushrooms cultivation, bioavailability.

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Conceptualization, Investigation, Writing - original draft, Writing - review & editing: Diene France de Souza.

Conceptualization, Writing - original draft, Writing - review & editing: Marliane de Cássia Soares da Silva.

Conceptualization, Writing - review & editing: Tainara Camila de Souza, Gabriel Cipriano Rocha, Maria Catarina Megumi Kasuya, Monique Renon Eller.

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INTRODUCTION

Mushrooms consist mainly of water, carbohydrates, such as β -glucans, and proteins [1]. Lipids, vitamins, minerals, and other compounds are present in lower concentrations, and therefore edible mushrooms can be good alternatives for low-calorie diets [1]. The main mushrooms cultivated worldwide are Champignon (*Agaricus bisporus*), which can have up to 35 % protein and 63 % carbohydrates on a dry basis, Shiitake (*Lentinula edodes*), with almost 18 % protein and 78 % carbohydrates, and the Oyster mushroom (*Pleurotus ostreatus*), which can reach 30 % protein and 82 % carbohydrates [1]. In general, these species can reach 4 % of lipids [1]. A diet rich in edible mushrooms also provides some compounds considered bioactive, such as phenolics and β -glucans, which increase the activity of antioxidant enzymes, reducing the concentration of free radicals, whose presence increases the occurrence of cancer, diabetes and cardiovascular diseases [1-2]. Thus, the consumption of mushrooms could prevent illnesses and also reduce cholesterol and glucose in blood [3-4]. In addition, compounds extracted from mushrooms presented antitumor activity [5-7].

The composition and, consequently, the benefits of mushrooms depend on the substrate in which they are cultivated [1]. Thus, the substrate can be enriched with minerals such as selenium, since mushrooms can absorb and convert inorganic forms of this mineral into more bioavailable organic forms, such as selenomethionine and selenocysteine [8]. Selenium is essential for the homeostasis of organisms, with a recommended daily intake of 55 μg for healthy adults [9]. This element contributes to molecular regeneration, reproduction, and embryonic development. Some of the most important selenoproteins for the organism are the iodothyronine-deiodinases, which metabolize thyroid hormones, the selenoprotein-P, responsible for selenium transport, and the antioxidant enzyme glutathione peroxidase (Gpx) [10]. Selenium deficiency affects people around the world, aggravating cardiovascular diseases, as well as diabetes *mellitus* and intestinal cancer [11]. Selenium deficiency is also associated with the onset of Keshan disease, characterized by heart failure of varying degrees, and Kashin-Beck disease, which can occur in pre- and adolescence, and is characterized by endemic osteoarthritis [12].

Selenium deficiency in soils in several regions of the world leads to the need for strategies of selenium supplementation. In these places, the intake of foods that would naturally be rich in selenium, such as broccoli, turnips, and radishes, may not be sufficient [11]. In this context, biofortification is an alternative to overcome this deficiency and could be done by adding selenium to the growing medium of foods such as potatoes [13], carrots [14], or edible mushrooms [15]. In this case, mushrooms present shorter cultivation time (approximately 30 days) [15] when compared to potatoes and carrots (112 and 120 days, respectively) [13-14]. When the

product of interest is the fungal mycelia, production time may be as little as seven days [16]. Moreover, mushroom cultivation is easy, cheap and may also be classified as sustainable, since it can be performed using agro-industrial by-products. Finally, the residues could still be used as fertilizers or for animal feed [17].

Biofortified edible mushrooms could provide dietary supplemental selenium, which is available mainly as organic forms such as selenomethionine and selenocysteine [8, 18], in addition to their nutritional and functional benefits [6-7]. Besides these forms, in mushrooms grown in the presence of selenium, it can be found combined with polysaccharides, in the form of selenopolysaccharides [7].

The enrichment of plants and mushrooms with minerals has been discussed in a recent review [19]. In this work the enrichment of mushrooms with selenium is reviewed, together with the limited information available regarding the selenium bioavailability and functionality in these foods. Thus, the present work aimed to gather relevant information focused on the effects of selenium on mushroom cultivation and properties, as well as the effects of its ingestion, helping to viabilize the production of selenium-enriched edible mushrooms and to stimulate their consumption, as functional foods.

Effect of selenium enrichment on the yield of edible mushrooms

Due to their ability to use different lignocellulosic substrates, mushrooms can be produced using different by-products [1]. In some cases, the product of interest may only be the mycelia, which is the first stage of mushroom production [16]. Naturally, they absorb and accumulate selenium from the substrate on which they develop, so the concentration of the mineral in the frutification bodies is generally low when they develop in substrates with low selenium content [20]. Therefore, it may be necessary to supply the substrates with selenium to produce enriched-mushrooms [15]. For this, different forms of selenium have been used. *P. ostreatus* and *P. djamor* mycelia were cultivated in the presence of sodium selenite (Na_2SeO_3) and selenate (Na_2SeO_4), respectively [16, 21], and sodium selenite were used in the substrate for cultivation of *P. ostreatus*, *P. eryngii* and *Calocybe indica* mushrooms [15, 22-24].

The use of selenium for mushroom enrichment can influence mushroom yield, as measured mainly by biological efficiency, which is how many grams of fresh mushroom are produced with each 100 g of dry substrate [15]. According to table 1, the use of selenium from sodium selenite and sodium selenate in coffee husks and red sorghum straw, respectively, increased the biological efficiency of *P. ostreatus* compared to cultivation on the unenriched substrate [15, 22]. On other hand, a reduction up to 50 % in biological efficiency was observed for *Calocybe indica* mushroom cultivated in wheat straw enriched with Na_2SeO_3 [23], and for *P.*

ostreatus and in *P. sajor-caju* mushrooms cultivated in wheat straw from seleniferous soil [25]. As observed in those studies, the use of selenium at low concentrations in the substrates for mushrooms cultivation can increase the biological efficiency until the presence of selenium affects the formation of fruiting bodies and reduces the biological efficiency. In *P. ostreatus* mushrooms grown in coffee husks enriched with 12.7 mg kg⁻¹ of selenium (Na₂SeO₃), for example, there was an increase of 20 % in biological efficiency, in comparison with mushrooms cultivated in non-enriched substrate [15].

Table 1 Effect of selenium addition in the yield of edible mushrooms

Mushroom species	Substrate	Se form	Se added in the substrate (mg kg ⁻¹)	BE (%)	Reference
<i>P. ostreatus</i>	Coffee husks	-	0.0	30.00	[15]
		Na ₂ SeO ₃	12.7	36.00	
<i>P. ostreatus</i>	Red sorghum straw	-	0.0	80.00	[22]
		Na ₂ SeO ₄	5.8	89.09	
		Na ₂ SeO ₄	17.5	93.33	
<i>Calocybe indica</i>	Wheat straw	-	0.0	72.90	[23]
		Na ₂ SeO ₃	20.0	51.06	
<i>P. ostreatus</i>	Wheat straw	Substrate from NSS	0.0	57.12	[25]
		Substrate from SS	40.5	26.07	
<i>P. sajor-caju</i>	Wheat straw	Substrate from NSS	0.0	57.65	
		Substrate from SS	40.5	30.46	

NSS = non-seleniferous soil. SS = Seleniferous soil. Se = Selenium. Na₂SeO₃ = Sodium selenite. Na₂SeO₄ = Sodium selenate. BE = Biological efficiency.

Absorption and conversion of inorganic to organic forms of selenium by mushrooms

The ability to absorb selenium from the substrate enriched with Na₂SeO₃ or Na₂SeO₄ has already been demonstrated for various species (Table 2). In addition to their ability to absorb the mineral, mushrooms convert inorganic to organic forms of selenium when grown on enriched substrates, accumulating this mineral in their mycelia and fruiting bodies [8, 26-29]. Sodium selenite is directly converted to hydrogen selenide, which is the metabolite firstly absorbed. However, when the medium is enriched with sodium selenate, its uptake of sodium selenate may be slower, since it is initially converted to sodium selenite and then to hydrogen selenide [26].

The mineral uptake and conversion capacity vary depending on the mushroom species, and the type and concentration of selenium in the substrate. The content of selenomethionine in *P. ostreatus* mushroom corresponded to 64.36 % of total selenium and, in the proteins extracted from this fungus, 88.41 % of the selenium was present in the form of these selenoamino acids [8]. The selenomethionine and selenocysteine

contents found in *P. ostreatus* mushroom corresponded to 27.13 and 6.47 % of total selenium, respectively [26]. In *Cordyceps militaris* mushroom, the selenocysteine and selenomethionine contents corresponded to approximately 15.00 and 25.00 % of total selenium, respectively [27]. In *Agrocybe aegerita* and in *H. erinaceus* mushrooms, the selenomethionine content was 80.45 and 55.67 % of total selenium, respectively [29].

Table 2 Absorption and conversion of selenium forms by fungi

Mushroom species	Substrate	Se form	Se added in the substrate (mg kg ⁻¹)	Se content in the mushrooms (mg kg ⁻¹)	SeMet (mg kg ⁻¹)	SeCys (mg kg ⁻¹)	Reference
<i>P. ostreatus</i>	CH	Na ₂ SeO ₃	25.4	267.55	172.2	-	[8]
Proteins from <i>P. ostreatus</i>	CH	Na ₂ SeO ₃	25.4	369.2	326.4	-	[8]
<i>P. ostreatus</i>	CH	Na ₂ SeO ₃	25.4	310.2	-	-	[15]
<i>P. ostreatus</i> mycelia	CM	Na ₂ SeO ₃	25.0	1015.4	-	-	[16]
<i>Calocybe indica</i>	WS	Na ₂ SeO ₃	20.0	14.7	-	-	[23]
<i>P. eryngii</i> mycelia	CM	Na ₂ SeO ₃	50.0	187.9	-	-	[24]
<i>P. eryngii</i>	WS	Na ₂ SeO ₃	50.0	13.6	-	-	[28]
<i>P. ostreatus</i>	CB	Na ₂ SeO ₃	25.0	179.1	-	-	[28]
<i>A. aegerita</i>	BS	Na ₂ SeO ₃	47.38	34.1	27.4	-	[29]
<i>H. erinaceus</i>		with Na ₂ SeO ₄		41.7	22.7	-	[29]
<i>P. ostreatus</i>	WC, CC, and RB	Na ₂ SeO ₃	5.0	44.2	12.0	2.9	[26]
<i>Cordyceps militaris</i>	CM	Na ₂ SeO ₃	40.0	129.8	32.8	21.5	[27]
		Na ₂ SeO ₄	40.0	134.7	35.4	17.8	[27]

CH = Coffee husks. CM = Culture medium. WS = Wheat straw. WC = Wood chip. CC = Corn cobs. CB = Sugarcane bagasse. BS = Beech sawdust. RB = Rice bran. Se = Selenium. Na₂SeO₃ = Sodium selenite. Na₂SeO₄ = Sodium selenate. SeMet = selenomethionine. SeCys = selenocysteine.

To evaluate selenium uptake capacity, some studies use the bioconcentration factor (BCF), calculated as the ratio between the mineral content in the fruiting bodies and in the substrate. The occurrence of bioconcentration is confirmed when BCF > 1 [29]. In *A. aegerita* and *H. erinaceus* mushrooms, the BCF of selenomethionine was 6.86 and 4.08, respectively, while for inorganic selenium it was 0.11 and 0.24 [29]. In *P. ostreatus* mushroom cultivated in wheat straw enriched with a mix of Na₂SeO₃ and Na₂SeO₄ at 0.25 mM, the BCF for selenomethionine, Na₂SeO₃ and Na₂SeO₄ was 2.07, 0.38 and 0.89, respectively. In this case, the selenomethionine content in fruiting bodies (50 µg g⁻¹) corresponded to 94.34 % of total selenium content (53 µg g⁻¹) [30]. This accumulation of selenomethionine, but not of inorganic forms of selenium, reflects the conversion of selenium from inorganic to organic forms by these species.

Chemical composition and antioxidant activity of enriched mushrooms

As has been demonstrated here, the composition of the substrate used for mushroom cultivation, the type and concentration of selenium added to this substrate as well as the mushroom species, influence their chemical composition and antioxidant activity.

In part, this occurs because selenium presents affinity with some minerals and, during mushrooms growing, its absorption by the fungal mycelia may increase their concentration in the fruiting bodies. This was demonstrated for calcium, magnesium, iron, zinc, and manganese in the genus *Pleurotus* spp., for example (Table 3) [24, 31-32]. However, the results are ambiguous, no patterns could be observed in this case, requiring further studies on the subject. Possibly, a great number of complex variables influence this matter, like the selenium form and concentration added, the type of substrate, the fungi and nutritional requirements of the mushrooms.

Table 3 Mineral ($\mu\text{g g}^{-1}$) composition of mushrooms cultivated on substrates added of selenium

Mushroom	Substrate	Se form	Se added (mg kg^{-1})	Ca	Mg	Fe	Zn	Mn	Reference
<i>P. djamor</i> mycelia	Culture medium	-	0.0	27.5	0.8	0.014	0.006	0.0	[21]
		Na_2SeO_3	12.5	17.5	1.0	0.024	0.005	0.0	
		Na_2SeO_4	12.5	23.4	1.4	0.030	0.007	0.0	
<i>P. eryngii</i> mycelia	Culture medium	-	0.0	618.0	1,750.0	314.0	151.0	99.8	[24]
		Na_2SeO_3	50.0	291.0	790.0	334.6	109.0	44.6	
<i>P. eryngii</i>	BS:WS (1:1)	-	0.0	174.0	1,170.0	38.5	49.4	9.0	[24]
		Na_2SeO_3	50.0	528.0	2,350.0	51.1	44.8	9.2	
<i>P. pulmonarius</i>	Corn cobs	-	0.0	56.7	30.7	0.3	0.7	0.1	[31]
		Na_2SeO_3	50.0	106.2	43.4	19.7	2.5	1.7	
<i>P. ostreatus</i>		-	0.0	17.8	25.6	21.5	4.2	3.1	[31]
		Na_2SeO_3	50.0	209.4	38.4	7.3	2.9	1.8	
<i>P. djamor</i>	CB, RB, and WB	-	0.0	31.0	2.7	108.0	105.0	13.7	[33]
		Na_2SeO_3	25.6	20.0	2.5	123.0	95.0	11.3	
<i>P. ostreatus</i>		-	0.0	34.0	1.6	57.0	46.0	6.3	[33]
		Na_2SeO_3	25.6	25.0	1.7	59.0	46.0	6.5	

BS = Beech sawdust. WS = Wheat straw. CB = Sugarcane bagasse. RB = Rice bran. WB = Wheat bran. Se = selenium. Na_2SeO_3 = Sodium selenite. Na_2SeO_4 = Sodium selenate. Ca = Calcium. Mg = Magnesium. Fe = Iron. Zn = Zinc. Mn = Manganese.

Selenium also has high affinity to albumins, globulins and glutelins, which are rich in glutamic acid, cysteine and methionine [33]. For this reason, the use of selenium in the growing substrate may increase the concentration of these amino acids in mushrooms. When it happens, the protein content may also be increased [23, 25, 30] (Table 4). For example, fruiting bodies of *Calocybe indica* produced using selenium (Na_2SeO_3) at

10.0 mg kg⁻¹ presented 71 % of increase in protein content, and there was also an increase in glutamic acid (from 2.9 to 4.7 %), cysteine (from 0.01 to 0.24 %), and methionine (from 0.15 to 0.27 %) contents [23].

Table 4 Protein content of mushrooms cultivated in substrates enriched with selenium

Mushroom	Substrate	Se form	Se added (mg kg ⁻¹)	CP	Reference
<i>Calocybe indica</i>	Wheat straw	-	0.0	14.33 %	[23]
		Na ₂ SeO ₃	10.0	25.31 %	
<i>P. florida</i>		Substrate from NSS	0.0	1.64 mg g ⁻¹	
		Substrate from SS	40.5	2.49 mg g ⁻¹	
<i>P. ostreatus</i>	Wheat straw	Substrate from NSS	0.0	1.73 mg g ⁻¹	[25]
		Substrate from SS	40.5	1.79 mg g ⁻¹	
<i>P. sajor-caju</i>		Substrate from NSS	0.0	2.49 mg g ⁻¹	
		Substrate from SS	40.5	2.64 mg g ⁻¹	
<i>P. ostreatus</i>		-	0.0	10.69 %	
		Na ₂ SeO ₃	50.0	18.21 %	
<i>P. pulmonarius</i>	Corn cobs	-	0.0	10.31 %	[31]
		Na ₂ SeO ₃	50.0	16.65 %	

NSS = Non-seleniferous soil. SS = Seleniferous soil. Se = selenium. Na₂SeO₃ = Sodium selenite. CP = Crude protein.

The synthesis of polysaccharides, such as β -glucans, can also be stimulated by the use of selenium in the substrate of cultivation. In fact, high concentrations of this mineral can be found in polysaccharides. In *Calocybe indica* mushrooms, most of the selenium was linked to proteins (4.80 $\mu\text{g g}^{-1}$) and polysaccharides (1.55 $\mu\text{g g}^{-1}$) in comparison with nucleic acid (0.13 $\mu\text{g g}^{-1}$) [23]. The use of selenium at 12.5 mg L⁻¹ (Na₂SeO₃) increased the protein (from 1.4 to 1.8 mg L⁻¹) and β -glucan (from 23.12 to 43.75 %) contents of *P. djamor* mycelia, in comparison with non-enriched culture medium [21]. On the other hand, *P. ostreatus* mushrooms cultivated in corn cobs enriched with selenium at 50 mg kg⁻¹ (Na₂SeO₃) presented reduced crude fiber content (from 20.12 to 10.40 %) [31]. Possibly, the high concentration used in this study negatively influenced the synthesis of fiber.

The addition of selenium (Na₂SeO₃) to the growing media can also increase the uptake and biosynthesis of micronutrients by the mushrooms, including several with functionality claims. The stress caused to the fungi when cultivated in Se-enriched substrates stimulates the biosynthesis of phenolics as a protective mechanism [2, 34] (Table 5). Some of these compounds are considered functional, like antioxidant compounds. Thus, the addition of selenium to the substrate to produce enriched mushrooms may increase the antioxidant capacity of these foods (Table 5), either by the higher uptake and biosynthesis of those compounds [35], or by the high content of organic forms of selenium. The use of selenium increased the content of phenolic compounds and

antioxidant activity in *P. ostreatus*, *P. eryngii*, *H. erinaceus*, *Ganoderma lucidum*, and *A. aegerita* mushrooms [2, 34]. However, the rise in the content of phenolics in *P. fossulatus* mushrooms cultivated in substrate from seleniferous soil did not significantly increase their antioxidant activity measured by DPPH [36]. In this case, the use of selenium (24.0 mg kg^{-1}) may have affected the uptake of other unmeasured compounds with antioxidant activity, such as proteins and fibers [34]. This parameter may also be related to the substrate on which the mushroom is cultivated [37].

Table 5 Influence of selenium addition to the cultivate substrate on the phenolic and antioxidant activity

Mushroom	Substrate	Se form	Se added	Total phenolic content (mg GAE g ⁻¹)	DPPH (% FRS)	Reference
<i>P. ostreatus</i>	BS	-	0.0 mM	6.69	41.5	[2]
		Na ₂ SeO ₃ with Na ₂ SeO ₄	0.5 mM	7.71	47.1	
<i>P. eryngii</i>		-	0.0 mM	4.44	37.4	
		Na ₂ SeO ₃ with Na ₂ SeO ₄	5.0 mM	7.18	52.9	
<i>H. erinaceus</i>	BS	-	0.0 mM	17.80	68.99	[34]
		Na ₂ SeO ₃ with Na ₂ SeO ₄	47.38 mM	27.00	84.63	
<i>Ganoderma Lucidum</i>		-	0.0 mM	29.00	50.93	
		Na ₂ SeO ₃ with Na ₂ SeO ₄	47.38 mM	40.00	86.99	
<i>Agrocybe Aegerita</i>		-	0.0 mM	17.00	74.16	
		Na ₂ SeO ₃ with Na ₂ SeO ₄	47.38 mM	21.00	93.56	
<i>P. fossulatus</i>	WS	Substrate from NSS	0.0 mg kg ⁻¹	6.45	36.0	[36]
		Substrate from SS	24.0 mg kg ⁻¹	7.10	40.6	

BS = Beech sawdust. WS = Wheat straw. Se = Selenium. Na₂SeO₃ = Sodium selenite. Na₂SeO₄ = Sodium selenate. GAE = Gallic acid equivalent. DPPH = 2,2-diphenyl-1-picryl hydrazyl radical. FRS = Free radical sequestration.

Bioaccessibility and functionality

Bioaccessibility is the fraction of a nutrient that is soluble in the gastrointestinal tract and becomes available for absorption by the enteric mucosa [11]. After absorption, the nutrient reaches the systemic circulation and is distributed to organs and tissues, becoming bioavailable, and subsequently, part of the nutrient may become bioactive [12]. Some studies describes the effects of selenized mushrooms extracts or the ingestion of these foods on selenium bioaccessibility, antitumor activity, and oxidative stress parameters such as Gpx, glutathione-

S-transferase (GST) activities, and malondialdehyde content (Tables 6 and 7). Selenium bioaccessibility is usually evaluated *in vitro* and it is influenced by the form of selenium added in substrate, and the mushroom processing, such as cooking (Table 6). In general, the gastrointestinal bioaccessibility is higher than gastric one [26, 38-39]. When the mushrooms are heated until boiling, the selenium in fruiting bodies generally decreases due to the leaching by the cooking water, as seen for *P. eryngii* mushrooms (from 17.65 to 12.71 $\mu\text{g g}^{-1}$). This process differently influences the selenium bioaccessibility, according to the selenium form added to the substrate and to the portion of the gastrointestinal tract evaluated [38]. Some studies have shown that the selenium present in enriched mushrooms is bioaccessible and is absorbed to become bioavailable (Table 6). This was demonstrated in rats plasma [18], and in calves serum [40], where selenium content increased when the animals were fed with selenized mushrooms [18, 40]. In addition, there were no changes in leukocytes, monocytes, and granulocytes of the calves [40].

The bioavailability of organic forms of selenium, predominantly found in enriched mushrooms [8, 26], may be higher than that of inorganic forms [18]. This could partially be attributed to the metabolism of these molecules. For example, sodium selenite is non-enzymatically converted to hydrogen selenide, while selenomethionine, when following a similar pathway, is converted enzymatically to selenocysteine and subsequently to hydrogen selenide [12]. Thus, the inorganic component is metabolized and excreted more rapidly than the organic forms of this mineral, so it is not available to be absorbed.

When selenium is absorbed, it becomes available to the metabolism. Increased antioxidant activity from selenium-enriched fungi has not only been demonstrated in mycelia and mushrooms, but also in tissues and plasma of animals fed with them (Table 6). The ingestion of selenized mushrooms increases the activity of glutathione peroxidase and glutathione-S-transferase [41-42], which may reduce the concentration of free radicals, partly responsible for the onset of cardiovascular diseases and cancers [1-2]. Additionally, it has been demonstrated that the ingestion of selenium-enriched mushrooms protects the intestinal barrier against stress caused by high body temperature (40 °C) [43]. In addition, the intake of solutions produced from selenized mushrooms or their derivatives increases the antioxidant activity in debilitated animals and reduces the damage caused by some diseases. For example, the Gpx activity increased in the liver and kidney of mice with induced diabetes fed with solutions of selenium-enriched mycelia from *Coprinus comatus* and *Catathelasma ventricosum* [3-4]. Additionally, their blood glucose decreased from nearly 20 to about 10 mM [3-4]. The same rise in Gpx activity was observed in the lung of mice with induced endotoxemia when they received an intraperitoneal injection of selenopolysaccharides, obtained from the mycelia of *Hypsizygus marmoreus* [44].

Table 6 Effects of the use of selenized mushrooms on bioaccessibility, bioavailability, and bioactivity

Bioaccessibility <i>in vitro</i> (%)						
Mushrooms	Se form	Se added (mg kg⁻¹)	Gastrointestinal	Gastric	Reference	
<i>P. ostreatus</i>	Na ₂ SeO ₃	5	91.89	73.78	[26]	
	Na ₂ SeO ₄		77.64	43.04		
	Se-yeast		82.34	63.05		
Uncooked <i>P. eryngii</i>	Na ₂ SeO ₃	5	78.40	70.30	[38]	
	Na ₂ SeO ₄		89.70	82.30		
	Se-yeast		80.00	76.30		
Cooked <i>P. eryngii</i>	Na ₂ SeO ₃		88.10	42.50	[39]	
	Na ₂ SeO ₄		89.00	68.40		
	Se-yeast		89.00	53.30		
<i>H. erinaceus</i>	Na ₂ SeO ₃	40	50.80	42.90	[39]	
Bioavailability <i>in vivo</i>						
Mushrooms	Se form	Treatment	Animal	Seric Se (µg L⁻¹)	Plasmatic Se (µg L⁻¹)	Reference
-	-	Control	Rats	-	291.00	[18]
<i>P. ostreatus</i>	Na ₂ SeO ₃	0.15 µg Se per g of diet		-	618.00	
-	Na ₂ SeO ₃			-	513.00	
-	-	Control	Calves	38.50	-	[40]
<i>L. edodes</i> Mycelia	Selol	5 µg of Se per kg ⁻¹ of b.w.		210.67	-	
Bioactivity <i>in vivo</i>						
Mushrooms	Se form	Treatment	Part of animal	Gpx (U mg ptn⁻¹)	GST (U mg ptn⁻¹)	Reference
<i>Coprinus comatus</i> mycelia	-	Control	Liver from	708.56		[3]
	Na ₂ SeO ₃	II 15.21 µg g ⁻¹	diabetic mices	773.86		
	-	Control	Kidney from	766.99		
<i>Catathelasma ventricosum</i> mycelia	Na ₂ SeO ₃	II 15.21 µg g ⁻¹	diabetic mices	852.19		[4]
	-	Control	Liver from	593.60		
	Na ₂ SeO ₃	OS 500 mg kg ⁻¹	diabetic mices	797.40		
<i>A. bisporus</i>	-	Control	Kidney from	400.30		[41]
	Na ₂ SeO ₃	OS 500 mg kg ⁻¹	diabetic mices	584.50		
	-	Control	Colon of rats	473.00	-	
<i>A. bisporus</i>	Na ₂ SeO ₃	1 µg of Se per g of diet		839.00	-	[42]
	-	Control	Liver of rats	-	606.90	
	Na ₂ SeO ₃	1 µg of Se per g of diet		-	717.24	
	-	Control	Breast of rats	-	33.49	
<i>Hypsizygus marmoreus</i>	Na ₂ SeO ₃	1 µg of Se per g of diet		-	46.05	[44]
	-	Control	Lung from mices	55.00	-	
	Na ₂ SeO ₃	II of SPS at 800 mg per kg ⁻¹ (b.w.)	with induced endotoxemia	125.00	-	

Se = Selenium. Na₂SeO₃ = Sodium selenite. Na₂SeO₄ = Sodium selenate. LDL = Low density lipoproteins. Se-yeast = selenized-yeast. Gpx = Glutathione peroxidase. GST = Glutathione-S-transferase. U = units. Ptn = Protein. b.w. = Body weight. Selol = Selenitriglycerides synthesized by Na₂SeO₃ with sunflower oil. OS = Oral solution. SPS = Selenopolysaccharides. II = Intraperitoneal injection.

Other studies have tested the antitumor activity of products derived from selenium-enriched mushrooms or their mycelia against various tumor cell lines. This activity varies depending on the cell line, mushroom species, and product extracted (Table 7). For example, the extract of selenium-enriched *Flammulina velutipes* caused low inhibition of development of cervical (HeLa) and colon (LS74) cancer cells, in comparison with extract of non-enriched mushroom [45]. However, selenopolysaccharides from *P. ostreatus* mushrooms reduced the viability of lung, and ovarian cancer cells [7], while *Auricularia auricula* extracts inhibited leukemic cancer cells [46]. Similarly, the viability of leukemic, breast, and cervical tumor cells was reduced when in contact with a solution of selenopolysaccharides from *G. lucidum* [5], while ovarian, lung and liver tumor cells were inhibited by selenopolysaccharides extracted from *Cordyceps gunnii* mycelia [47].

Table 7 Effects of the use of selenized mushrooms or their derivatives on the *in vitro* antitumor activity

Mushrooms	Se form	Se added	Product	Culture Cells	Inhibition	Unit of inhibition	Reference
<i>G. lucidum</i> mycelia	-	0.0 mg L ⁻¹	SPS	K-562	2.10	IC ₅₀ (μM)	[5]
	Na ₂ SeO ₃	400.0 mg L ⁻¹			0.11		
	-	0.0 mg L ⁻¹		MCF-7	1.26		
	Na ₂ SeO ₃	400.0 mg L ⁻¹			0.14		
	-	0.0 mg L ⁻¹		HeLa	1.58		
<i>P. ostreatus</i>	Na ₂ SeO ₃	400.0 mg L ⁻¹			0.17		
	-	0.0 μg L ⁻¹	SPS	A549	0.00	%	[7]
	Na ₂ SeO ₃	90.0 μg mL ⁻¹	600 μg mL ⁻¹		48.30		
	-	0.0 μg L ⁻¹		SKOV3	0.00		
	Na ₂ SeO ₃	90.0 μg mL ⁻¹			41.20		
-	0.0 mg L ⁻¹	Ethanollic extract	HeLa	259.69	IC ₅₀ (μg mL ⁻¹)		
Na ₂ SeO ₃	1.3 mg L ⁻¹			331.91			
-	0.0 mg L ⁻¹		LS74	338.47			
<i>F. velutipes</i> mycelia	Na ₂ SeO ₃	1.3 mg L ⁻¹			348.46		
	-	0.0 mM	SPS	HL60	0.00	%	[46]
	Na ₂ SeO ₃	100.0 mM	200 μg mL ⁻¹		80.00		
-	0.0 mM		U937	5.88			
<i>Auricularia auricula</i>	Na ₂ SeO ₃	100.0 mM			80.00		
	-	0.0 mg L ⁻¹	SPS	SKOV-3	3.33	%	[47]
	Na ₂ SeO ₃	7.0 μg mL ⁻¹	400 μg mL ⁻¹		70.00		
-	0.0 mg L ⁻¹		H1299	5.66			
<i>Cordyceps Gunnii</i> mycelia	Na ₂ SeO ₃	7.0 μg mL ⁻¹			64.00		
	-	0.0 mg L ⁻¹		HepG2	7.14		
	Na ₂ SeO ₃	7.0 μg mL ⁻¹			67.50		
	-	0.0 mg L ⁻¹					

Se = Selenium. Na₂SeO₃ = Sodium selenite. b.w. = Body weight. SPS = Selenopolysaccharides. U937, HL60, K-562 = Leukemic cell lines. H1299, A549 = Lung tumor cell lines. HeLa, LS74, SKOV-3, HepG2, MCF-7, AGS = Cervical, colon, ovarian, liver, breast, gastric tumor cell lines.

The antitumor activity of selenium-enriched mushrooms was also demonstrated *in vivo*, with rats and mice that were inoculated with ovarian (SKOV-3) or gastric (MGC-803) tumor cells. The tumor size significantly decreased after the animals had received intragastric or intravenous injections of a solution at 400 $\mu\text{L kg}^{-1}$ or 2,500 $\mu\text{g kg}^{-1}$ of selenopolysaccharides [6, 47].

Conclusions

The addition of selenium, at limit concentrations, to the substrate of cultivation of fungi increases the biological efficiency, protein content and antioxidant activity of the mushrooms, which can favour the cultivation of edible selenium-enriched mushrooms on agroindustrial by-products. Independently of which form is provided, fungi are able to convert inorganic forms of selenium in organic ones, thus most of the selenium found in selenized mushrooms is present in the organic form, which is more bioavailable. The selenium from the mushrooms was found to be bioaccessible, bioavailable and bioactive, since *in vitro* and *in vivo* experiments demonstrated the antitumor activity of extracts and molecules derived from these foods. Thus, edible mushrooms should be considered as vectors for selenium supplementation in human diets.

References

1. Chang ST, Miles PG (2004) Mushrooms: cultivation, nutritional value medicinal effect and environmental impact. CRC Press, Boca Raton
2. Gąsecka M, Mleczek M, Siwulski M et al (2015) The effect of selenium on phenolics and flavonoids in selected edible white rot fungi. *LWT - Food Science and Technology* 63:726–731.
<http://dx.doi.org/10.1016/j.lwt.2015.03.046>
3. Yu J, Cui P, Zeng W et al (2009) Protective effect of selenium-polysaccharides from the mycelia of *Coprinus comatus* on alloxan-induced oxidative stress in mice. *Food Chemistry* 117:42–47.
<https://doi.org/10.1016/j.foodchem.2009.03.073>
4. Liu Y, Li C, Luo X et al (2015) Characterization of selenium-enriched mycelia of *Catathelasma ventricosum* and their antihyperglycemic and antioxidant properties. *Journal of Agricultural and Food Chemistry* 63:562–568. <https://doi.org/10.1021/jf5050316>
5. Shang D, Zhang J, Wen L et al (2009) Preparation, characterization, and antiproliferative activities of the se-containing polysaccharide SeGLP-2B-1 from Se-Enriched *Ganoderma lucidum*. *Journal of Agricultural and Food Chemistry* 57:7737–7742. <https://doi.org/10.1021/jf9019344>
6. Zeng D, Zhao J, Luk K et al (2019) Potentiation of *in vivo* anticancer efficacy of selenium nanoparticles by mushroom polysaccharides surface decoration. *Journal of Agricultural and Food Chemistry* 67:2865–2876. <https://doi.org/10.1021/acs.jafc.9b00193>
7. Zhang Z, Zhang Y, Liu H et al (2021) A water-soluble selenium-enriched polysaccharide produced by *Pleurotus ostreatus*: Purification, characterization, antioxidant and antitumor activities *in vitro*. *International Journal of Biological Macromolecules* 168:356–370.
<https://doi.org/10.1016/j.ijbiomac.2020.12.070>
8. Assunção LS, Da Silva MCS, Fernandez MG et al (2014) Speciation of selenium in *Pleurotus ostreatus* and *Lentinula edodes* mushrooms. *Journal of Biotechnology Letters* 5:79–86.
<http://hdl.handle.net/10272/11489>
9. Institute of Medicine (2000) Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC, Academic Press
10. Beckett GJ, Arthur JR (2005) Selenium and endocrine systems. *Journal of Endocrinology* 184:455–465.
<https://doi.org/10.1677/joe.1.05971>

11. Prauchner CA (2014) A Importância do Selênio para a Agropecuária e Saúde Humana. UFSM, Santa Maria
12. Thiry C, Ruttens A, Temmerman L et al (2012) Current knowledge in species-related bioavailability of selenium in food. *Food Chemistry* 130:767-784. <https://doi.org/10.1016/j.foodchem.2011.07.102>
13. Turakainen M, Hartikainen H, Sarjala T (2008) Impact of selenium enrichment on seed potato tubers. *Agricultural and Food Science* 17:278-288. <https://doi.org/10.2137/145960608786118802>
14. Kápolna E, Hillestrøm PR, Laursen KH et al (2009) Effect of foliar application of selenium on its uptake and speciation in carrot. *Food Chemistry* 115:1357-1363. <https://doi.org/10.1016/j.foodchem.2009.01.054>
15. Da Silva MCS, Naozuka J, Luz JMR et al (2012) Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chemistry* 131:558-563. <https://doi.org/10.1016/j.foodchem.2011.09.023>
16. Da Silva MCS, Luz JMR, Paiva APS et al (2019) Growth and tolerance of *Pleurotus ostreatus* at different selenium forms. *Journal of Agricultural Science* 11:151-158. <https://doi.org/10.5539/jas.v11n2p151>
17. Kasuya MCM, Luz JMR, Nunes MD et al (2015) Production of selenium-enriched mushrooms in coffee husks and use of this colonized residue. In: Preedy VR (ed) *Coffee in health and disease prevention*, Elsevier, London, pp 301–309
18. Da Silva MCS, Naozuka J, Oliveira PV et al (2010) *In vivo* bioavailability of selenium in enriched *Pleurotus ostreatus* mushrooms. *Metallomics* 2:162-166. <https://doi.org/10.1039/B915780H>
19. Oliveira AP, Naozuka J (2020) Enriquecimento elementar por meio de cultivo: plantas e cogumelos. *Química Nova* 43:1277-1293. <http://dx.doi.org/10.21577/0100-4042.20170601>
20. Falandysz J (2008) Selenium in edible mushrooms. *Journal of Environmental Science and Health, Part C* 26:256–299. <https://doi.org/10.1080/10590500802350086>
21. Velez MEV, Luz JMR, Da Silva MCS et al (2019) Production of bioactive compounds by the mycelial growth of *Pleurotus djamor* in whey powder enriched with selenium. *LWT- Food Science and Technology* 114:1-9. <https://doi.org/10.1016/j.lwt.2019.108376>
22. Carrasco-Gonzalez JA, Serna-Saldívar SO, Gutierrez-Urbe JA (2017) Mycochemical changes induced by selenium enrichment in *P. ostreatus* fruiting bodies. *Journal of Agricultural and Food Chemistry* 65:4074-4082. <https://doi.org/10.1021/acs.jafc.7b00715>

23. Rathore H, Sharma A, Prasad S et al (2018) Selenium bioaccumulation and associated nutraceutical properties in *Calocybe indica* mushroom cultivated on Se-enriched wheat straw. *Journal of Bioscience and Bioengineering* 126:1-6. <https://doi.org/10.1016/j.jbiosc.2018.04.010>
24. Zięba P, Kała K, Włodarczyk A et al (2020) Selenium and zinc biofortification of *Pleurotus eryngii* mycelium and fruiting bodies as a tool for controlling their biological activity. *Molecules* 25:1-18. <https://doi.org/10.3390/molecules25040889>
25. Kaur G, Kalia A, Sodhi HS (2017) Selenium biofortification of *Pleurotus* species and its effect on yield, phytochemical profiles, and protein chemistry of fruiting bodies. *Journal of Food Biochemistry* 42:1-7. <https://doi.org/10.1111/jfbc.12467>
26. Zhou F, Dinh QT, Yang, W et al (2019) Assessment of speciation and *in vitro* bioaccessibility of selenium in Se-enriched *Pleurotus ostreatus* and potential health risks. *Ecotoxicology and Environmental Safety* 185:1-9. <https://doi.org/10.1016/j.ecoenv.2019.109675>
27. Hu T, Zhao G, Wu W et al (2018) Selenium biofortification and antioxidant activity in *Cordyceps militaris* supplied with selenate, selenite, or selenomethionine. *Biological Trace Element Research* 2019:553-561. <https://doi.org/10.1007/s12011-018-1386-y>
28. Souza DF, Da Silva MCS, Alves MP et al (2021) By-products as substrates for production of selenium-enriched *Pleurotus ostreatus* mushrooms. *Waste and Biomass Valorization*. <https://doi.org/10.1007/s12649-021-01586-9>
29. Niedzielski P, Mleczek M, Siwulski M et al (2014) Efficacy of supplementation of selected medicinal mushrooms with inorganic selenium salts. *Journal of Environmental Science and Health, Part B* 49:929-937. <https://doi.org/10.1080/03601234.2014.951576>
30. Niedzielski P, Mleczek M, Siwulski M et al (2015) Supplementation of cultivated mushroom species with selenium: bioaccumulation and speciation study. *European Food Research and Technology* 241:419-426. <https://doi:10.1007/s00217-015-2474-2>
31. Fasoranti OF, Ogidi CO, Oyetayo VO et al (2019) Nutrient contents and antioxidant properties of *Pleurotus* spp. cultivated on substrate fortified with selenium. *Current Research in Environmental and Applied Mycology* 9:66-76. <https://doi.org/10.5943/cream/9/1/7>
32. Oliveira AP, Leme FO, Nomura CS et al (2019) Elemental imaging by Laser induced breakdown spectroscopy to evaluate selenium enrichment effects in edible mushrooms. *Nature* 9:1-10. <https://doi.org/10.1038/s41598-019-47338-7>

33. Oliveira AP, Naozuka J (2019) Preliminary results on the feasibility of producing selenium-enriched pink (*Pleurotus djamor*) and white (*Pleurotus ostreatus*) oyster mushrooms: Bioaccumulation, bioaccessibility, and Se-proteins distribution. *Microchemical Journal* 145:1143-1150. <https://doi.org/10.1016/j.microc.2018.12.046>
34. Gąsecka M, Mleczek M, Siwulski M et al (2016) Phenolic and flavonoid content in *Hericium erinaceus*, *Ganoderma lucidum*, and *Agrocybe aegerita* under selenium addition. *Acta alimentaria* 45:300-308. <https://doi.org/10.1556/066.2016.45.2.18>
35. Arteel GE, Sies H (2001) The biochemistry of selenium and the glutathione system. *Environmental Toxicology and Pharmacology* 10:153-158. [https://doi.org/10.1016/s1382-6689\(01\)00078-3](https://doi.org/10.1016/s1382-6689(01)00078-3)
36. Bhatia P, Bansal C, Prakash R et al (2014) Selenium uptake and associated anti-oxidant properties in *Pleurotus fossulatus* cultivated on wheat straw from seleniferous fields. *Acta Alimentaria* 43:280-287. <https://doi.org/10.1556/AAlim.43.2014.2.12>
37. Frankel EN, Meyer AS (2000) The problem of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *Journal of the Science of Food and Agriculture* 80:1925-1941. [https://doi.org/10.1002/1097-0010\(200010\)80:13<1925::AID-JSFA714>3.0.CO;2-4](https://doi.org/10.1002/1097-0010(200010)80:13<1925::AID-JSFA714>3.0.CO;2-4)
38. Zhou F, Peng Q, Wang M et al (2021) Influence of processing methods and exogenous selenium species on the content and *in vitro* bioaccessibility of selenium in *Pleurotus eryngii*. *Food Chemistry* 338:1-8. <https://doi.org/10.1016/j.foodchem.2020.127661>
39. Hu T, Hui G, Li H et al (2020) Selenium biofortification in *Hericium erinaceus* (Lion's Mane mushroom) and its *in vitro* bioaccessibility. *Food Chemistry* 331:1-9. <https://doi.org/10.1016/j.foodchem.2020.127287>
40. Muszyńska B, Szacawa E, Bederska-Łojewska D et al (2020) Preliminary study on Se-enriched *Lentinula edodes* mycelium as a proposal of new feed additive in selenium deficiency. *Plos One* 15:1-19. <https://doi.org/10.1371/journal.pone.0233456>
41. Maseko T, Howell K, Dunshea FR et al (2014) Selenium-enriched *Agaricus bisporus* increases expression and activity of glutathione peroxidase-1 and expression of glutathione peroxidase-2 in rat colon. *Food Chemistry* 146:327-333. <https://doi.org/10.1016/j.foodchem.2013.09.074>
42. Spolar MR, Schaffer EM, Beelman RB et al (1999) Selenium-enriched *Agaricus bisporus* mushrooms suppress 7,12 dimethylbenz[a]anthracene bioactivation in mammary tissue. *Cancer Letters* 138:145-150. [https://doi.org/10.1016/S0304-3835\(99\)00003-8](https://doi.org/10.1016/S0304-3835(99)00003-8)

43. Maseko T, Dunshea FR, Howell K et al (2014) Selenium-enriched *Agaricus bisporus* mushroom protects against increase in gut permeability *ex vivo* and up-regulates glutathione peroxidase 1 and 2 in hyperthermally-induced oxidative stress in rats. *Nutrients* 6:2478-2492.
<https://doi.org/10.3390/nu6062478>
44. Liu M, Yao W, Zhu Y et al (2018) Characterization, antioxidant and antiinflammation of mycelia selenium polysaccharides from *Hypsizygus marmoreus* SK-03. *Carbohydrate Polymer* 201:566-574.
<https://doi.org/10.1016/j.carbpol.2018.08.099>
45. Milovanovic T, Stanojkovic T, Stajic M et al (2015) Se effect on biological activity of *Flammulina velutipes*. *Italian Journal of Food Science* 27:1-7. <https://doi.org/10.14674/1120-1770/ijfs.v74>
46. Jin Y, Cai L, Yang O et al (2020) Anti-leukemia activities of selenium nanoparticles embedded in nanotube consisted of triple-helix β -D-glucan. *Carbohydrate Polymers* 240:1-10.
<https://doi.org/10.1016/j.carbpol.2020.116329>
47. Sun H, Zhu Z, Tang Y et al (2018) Structural characterization and antitumor activity of a novel Se-polysaccharide from selenium-enriched *Cordyceps gunnii*. *Food & Function* 9:2744-2754.
<https://doi.org/10.1039/c8fo00027a>

CAPÍTULO 2: By-products as substrates for production of selenium-enriched *Pleurotus*

ostreatus mushrooms

Artigo publicado na revista

Waste and Biomass Valorization



By-Products as Substrates for Production of Selenium-Enriched *Pleurotus ostreatus* Mushrooms

Diene France de Souza¹ · Marliane de Cássia Soares da Silva² · Maiane de Paula Alves² · Dairon Pérez Fuentes³ · Lucas Eduardo Oliveira Porto³ · Pedro Vitoriano de Oliveira³ · Maria Catarina Megumi Kasuya² · Monique Renon Eller¹ 

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Abstract

The use of by-products reduces costs for the cultivation of Se-enriched mushrooms, whose consumption could reduce problems associated with Se deficiency. Treatments to decrease by-products contamination, such as sterilization, are also expensive and thus are not accessible for small producers. In this way, this work aimed to evaluate the influence of Se at 25 mg kg⁻¹ (sodium selenite) on the growth, composition, and antioxidant activity of *Pleurotus ostreatus* mushrooms cultivated on substrates composed of sugarcane bagasse (CB) and spent brewer's grain (BSG) treated with a hydrated lime solution. The use of sodium selenite did not affect the biological efficiency (33.79–52.31 g 100 g⁻¹), antioxidant activity (32.79–44.67%), and phenolic content (458.78–754.96 mg EAG 100 g⁻¹) of mushrooms, which had high concentrations of protein (17.07–23.50%), fibers (11.13–13.32%) and ash (4.46–7.21%). The mushrooms grown on CB alone presented 179.07 μg g⁻¹ of Se, so the consumption of 0.31 g of dry mushroom would be sufficient to achieve the recommended daily intake of Se. The CB treated with the hydrated lime solution can be used as an inexpensive and efficient substrate to produce Se-enriched *P. ostreatus* mushrooms.

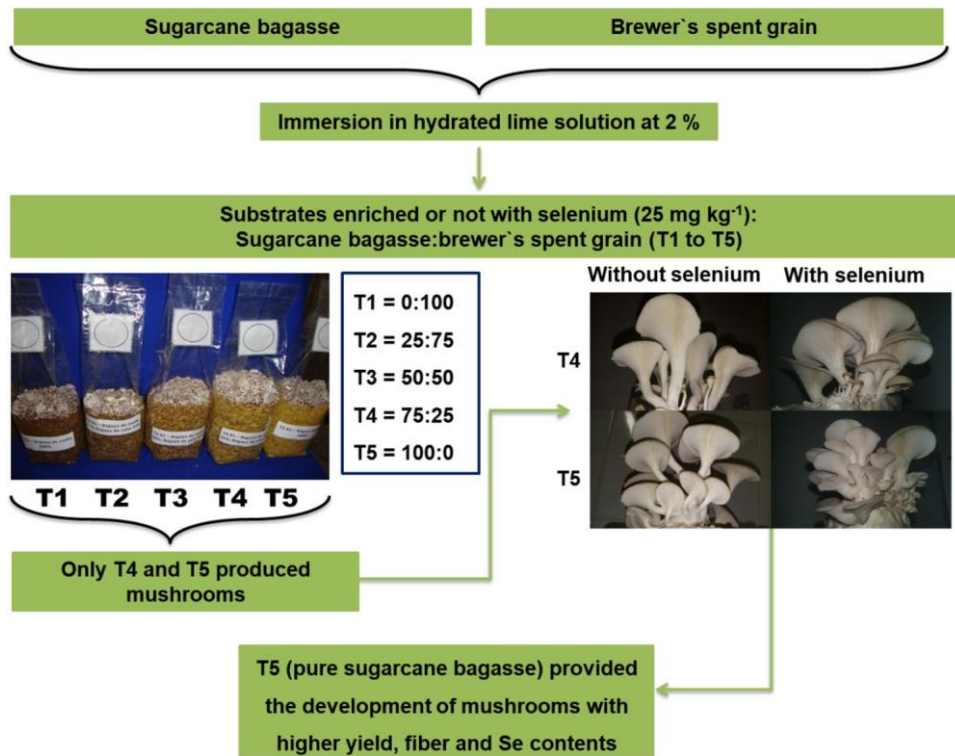
✉ Monique Renon Eller
monique.eller@ufv.br

¹ Department of Food Technology, Universidade Federal de Viçosa (UFV), Viçosa, MG 36570-900, Brazil

² Department of Microbiology, Universidade Federal de Viçosa (UFV), Viçosa, MG 36570-900, Brazil

³ Institute of Chemistry, Universidade de São Paulo (USP), São Paulo, SP 05508-900, Brazil

Graphic Abstract



Keywords Mushroom cultivation · Sodium selenite · Sugarcane bagasse · Added value products · Hydrated lime

Statement of Novelty

Fortification of mushrooms with Se aimed to produce a nutritionally rich food that provides the complementary Se to the diet. This study showed that it is possible to produce Se-enriched mushrooms in pure sugarcane bagasse or combined with brewer's spent grain treated by hydrated lime solution, an inexpensive treatment for substrate disinfection. This study also showed that some variables, such as biological efficiency and protein and mineral contents, are differently influenced by Se enrichment according to the substrate used.

Introduction

Brazil is the world's largest sugarcane producer [1] and one of the largest beer-producing countries [2]. For each ton of sugarcane processed, 270 to 290 kg of bagasse (CB) is generated [3], while 100 L of beer generates 14 to 20 kg of brewer's spent grain (BSG) [4]. The inappropriate discard or storage of these by-products impacts the

environment causing acidification and eutrophication of water bodies and soil pollution [3, 5–7]. These by-products can be transformed to generate incomes [3, 8] through the production of products such as bioethanol, biodegradable plastics, or even food, such as edible mushrooms [3, 9–11]. For mushroom production, the substrate must be disinfected, with sterilization being the most common treatment [12], although it is expensive and often inaccessible to small producers [13, 14]. For this reason, the process of immersing the substrate in an alkaline solution has been considered an alternative process [13–15]. Immersion of substrates such as CB, grass, stover, and corncob in an alkaline solution for at least 4 h is sufficient for disinfection for the production of some mushroom species [15–18]. Treatment with hydrated lime solution must be tested for other by-products as an alternative to producing mushrooms.

From 45,000 species of mushrooms in the world, 2000 are considered edible [19] and *Pleurotus ostreatus* is one of the most relevant, since it easily adapts to various cultivation methods and substrates and has high nutritional value [20]. Edible mushrooms are rich in fiber, protein, vitamins, and minerals, and have low lipid content, and can be used in low-calorie diets [21]. Besides their nutritional benefits,

some β -glucans found in mushrooms have antitumor activity [22]. However, the substrate used for fungi cultivation influences the nutritional content of the mushrooms, since they absorb and transform nutrients from the medium in which they are grown [23]. In this way, the by-product used to cultivate mushrooms, such as *P. ostreatus*, can be added with minerals such as selenium (Se) in order to produce mushrooms enriched with this element [17, 24–26]. Several soils in the world are deficient in selenium [27], which is essential for the activity of enzymes such as glutathione-peroxidase, selenoprotein-P, and iodothyronine-deiodinases, responsible for antioxidant activity, Se transport, and conversion of thyroid hormones in the human organism, respectively [28]. Thus, Se deficiency can cause thyroid dysfunctions, skeletal muscular dystrophy, depigmentation of hair and skin, and cardiac disorders [27–29].

For the cultivation of Se-enriched mushrooms, sodium selenite (Na_2SeO_3) or selenate (Na_2SeO_4) have been used. Na_2SeO_3 is cheaper and more bioavailable because it is directly converted to hydrogen selenide (H_2Se), which is the initial metabolite of the uptake, while Na_2SeO_4 is first converted to Na_2SeO_3 , to be further converted to H_2Se [27, 30–33]. Both forms of Se have already been added to mushroom growing medium [24, 25, 34–38] or in their heat shock water [39]. By-products such as coffee husks [24], CB [25], and corncob [40] have already been used for the cultivation of Se-enriched mushrooms. However, none of this research used alkaline immersion to disinfect the raw materials.

Thus, this study aimed to evaluate the yield, composition, and antioxidant activity of Se-enriched *P. ostreatus* mushrooms grown on substrates composed of combinations of CB and BSG pre-treated in alkaline solution.

Materials and Methods

Microorganism and Spawn Production

The isolate *P. ostreatus* PLO 02 was obtained from the fungal collection of the Laboratory of Mycorrhizal Associations, Department of Microbiology/BIOAGRO, in the Universidade Federal de Viçosa, Minas Gerais, Brazil. For mycelia production, the isolate was cultivated in a Petri dish containing potato dextrose agar (PDA, Merck, Darmstadt, Germany), pH 5.8, and incubated at 25 °C. After seven days, four disks of the PDA containing mycelia were used for spawn production on 100 g of sterilized sorghum grains [13].

Preparation of Substrates

Sugarcane bagasse (CB) and brewer's spent grain (BSG) were obtained from local producers of Viçosa, Minas Gerais, Brazil. CB was previously dried and crushed into

pieces of ~ 2 cm. Both the by-products were immersed in a hydrated lime (Engecal, Almirante Tamandaré, Brazil) solution ($\text{Ca}(\text{OH})_2$) at 2% (m v^{-1}) for 16 h and centrifuged for 1 min at 600 rpm (50.4 g) for the removal of excess solution, as previously standardized [41, 42].

Determination of pH, Proximate and Mineral Analysis of By-Products

The pH, proximate and mineral compositions of the CB and BSG were determined before and after their immersion in the $\text{Ca}(\text{OH})_2$ solution. The pH was monitored according to AOAC [43]. For this, 5 g of each material was homogenized with 50 mL of distilled water for 30 min and the pH (pHmeter Kasvi model K39-1014B) of the supernatant was measured. Total crude protein content was determined by the Kjeldahl method using 6.25 and 5.83 as the conversion factors for CB and BSG, respectively [44]. The fat was measured by Soxhlet extraction with petroleum ether (Merck, Darmstadt, Germany), and ash content by gravimetry [45]. The crude fiber content was calculated after the samples were submitted to acid and basic digestions, and then, to incineration [46].

For Se quantification, an aliquot of 10 g of each substrate was ground in a cryogenic mill (Marconi, Brazil) with a self-contained liquid nitrogen bath. After 5 min of pre-cooling, 5 grinding cycles of 2 min each were employed, followed by a re-cooling stage of 1 min between cycles by switching off the magnetic field. Masses around 150 mg of ground substrates were digested in a microwave oven (Anton Paar, Multiwave 3000), using a diluted acid mixture of 3.0 ml nitric acid (HNO_3) + 1.0 ml hydroxide peroxide (H_2O_2) (both purchased from Merck, Darmstadt, Germany) + 2.0 ml H_2O and the following heating program (step, temperature/°C, ramp/min, hold/min): 1, 140, 5, 1; 2, 180, 4, 5; and 3, 200, 4, 10. The Se quantification was carried out with an atomic absorption spectrometer (AnalytikjenaAG, ZEE nit 60), equipped with a transversely heated graphite atomizer, pyrolytically coated graphite tube, and transversal Zeeman-effect background corrector. The spectrometer was operated with a hollow cathode lamp, using 10 μL of samples or analytical calibration solutions (5 to 90 $\mu\text{g L}^{-1}$ in 1.0 M HNO_3). Argon 99.998% (v v^{-1}) (Air Liquide Brasil, Brazil) was used as protective and purge gas. The heating program used for the graphite furnace was the following (step, temperature/°C, ramp/s, hold/s): drying I, 100, 10, 15; drying II, 130, 10, 20; pyrolysis, 1200, 100, 20; atomization, 2300, 0, 5; and cleaning 2500, 1, 2. In all steps, Argon flow rate was 250 ml min^{-1} , except during atomization that was interrupted [47]. The results were expressed as micrograms of Se per g of substrate.

The determination of other minerals was performed using 500 mg of each substrate digested with 5 mL of a

nitro-perchloric (Sigma-Aldrich, Darmstadt, Germany) acid mixture (7:3) [48] in a digester block, at temperatures ranging from 130 to 250 °C. Calcium (Ca), magnesium (Mg), and iron (Fe) were measured by an atomic absorption spectrometer (Varian, Spectra A 20). Potassium (K) and sodium (Na) were measured by photometry with flame emission (Micronal, 462). Phosphorus (P) was determined by the colorimetric method at 725 nm (CELM, E-225 D) [49]. The results were expressed as mg of mineral per g of substrate. For the equipment calibration, analytical calibration solutions were prepared.

Experimental Design and Statistical Analysis

The experiment was carried out with a completely randomized design (CRD), with 5 substrates and 2 levels of Se concentration (with or without), at a 5 × 2 factorial scheme. The substrates consisted of different proportions of CB:BSG at 0:100 (T1); 25:75 (T2); 50:50 (T3); 75:25 (T4) and 100:0 (T5). Five repetitions (bags) were carried out per substrate, totaling 50 experimental units. When applied, Se was provided as sodium selenite (Na₂SeO₃) (Vetec, Duque de Caxias, Brazil) at 25 mg kg⁻¹. The data were submitted to analysis of variance (ANOVA), followed by Tukey test (5%), when necessary. For comparison of each by-product before and after immersion in hydrated lime solution, a T-test was used. Pearson's correlation analysis was performed between Se content in mushrooms and their mineral composition, antioxidant activity, and phenolic content. These statistical analyses used the R software (version 4.0.4). A Principal component analysis (PCA) [50] was carried out to demonstrate the influence of Na₂SeO₃ on the mushrooms characteristics: proximate and mineral composition, antioxidant activity and phenolic content. The software Chemoface (version 1.64) [51] was used for PCA analysis, and the data were initially treated by autoscaling.

Production of Mushrooms

CB and BSG were mixed at the proportions of each treatment and 500 g were placed into polypropylene bags with 10 mL of distilled water or sodium selenite solution that provided 25 mg kg⁻¹ of Se per bag [24]. This single concentration was chosen because previous work showed that it is the maximum yielding concentration without causing unpleasant odors and deformities in the fruiting bodies [17, 24]. At the end, the substrates were inoculated with 50 g of the fungus spawn and the bags were sealed and incubated at 25 °C until the complete colonization of the substrates by the fungal mycelium. The fruiting was induced at 10 °C for 24 h and mushroom production occurred at 23 °C and 80% RU. When the primordia appeared, the tops of the bags were cut with scissors and

removed. Harvesting was performed only once when the fruiting bodies had completed their development. The mushrooms of each treatment were evaluated.

For the mushrooms yield, the following data were analyzed: days after inoculation until harvest (d), fresh weight (g), dry weight (g), and biological efficiency (BE). BE was calculated as the percentage of the weight of fresh mushrooms in relation to the dry weight of the substrate [52]. The mushrooms were dehydrated in a food dehydrator with forced air circulation at 55 °C (Meloni[®], Pratic Dryer) until the weight was constant and then ground in a mini food processor (Palcookin[®], TK102), for dry weight (g) and for further analysis as described below.

The mushrooms were analyzed for proximate composition and for the amounts of selenium (Se), calcium (Ca), magnesium (Mg), iron (Fe), potassium (K), sodium (Na), and phosphorus (P), as described previously. The total crude protein content was calculated using the conversion factor 4.38 [53].

Antioxidant Activity and Phenolic Content of Mushrooms

Sample Preparation

Samples of 250 mg of dry and ground mushrooms or positive control—butylated hydroxytoluene (BHT) (Sigma-Aldrich, Darmstadt, Germany)—were subjected to three consecutive extractions using 5 mL of methanol (Synth, Diadema, Brazil) solution 80% (v v⁻¹) on an orbital shaker (Nova Ética[®], model 109) at 150 rpm for 2 h. After each extraction, the supernatant was removed, and, in the end, the three supernatants were homogenized. The extract was filtered and the volume was completed to 15 mL with methanol 80% (v v⁻¹) [54].

Antioxidant Activity of Mushrooms

The antioxidant activity of the mushrooms was measured by the 2,2-diphenyl-1-picryl hydrazyl radical (DPPH) and 2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) methods, both reagents were purchased from Sigma-Aldrich (Darmstadt, Germany). First, 7.5 µL of mushroom extract or BHT were added to 292.5 µL DPPH solution at 60 µM on microplate wells [55]. Methanol 80% (v v⁻¹) was used as control. Readings were carried out 110 min later (stabilization time) at a wavelength of 517 nm (Multiskan GO[®]—Thermo Scientific), and the results were expressed as the percentage of free radical sequestration (% FRS), according to the Eq. 1:

$$\% \text{FRS} = (Ac - Aa) \times 100 Aa^{-1} \quad (1)$$

where Ac is control absorbance and Aa is sample absorbance.

The antioxidant activity determined by ABTS radical was performed by a traditional method, with some modifications [56]. The working solution was obtained by adding 17.6 μL of potassium persulfate (Synth, Diadema, Brazil) solution (140 mM) to 1 mL of an ABTS radical solution at 7 mM; ethanol (Sigma-Aldrich, Darmstadt, Germany) was added to this solution until the absorbance reached 0.70 ± 0.05 at 734 nm. Then, 2.8 μL of five dilutions of each extract were added to 280 μL of working solution on microplate wells, and the absorbance was observed at 734 nm (Multiskan GO[®]—Thermo Scientific) after 30 min. Trolox (Sigma-Aldrich, Darmstadt, Germany) was used as a reference and the results were expressed as μM of Trolox equivalent antioxidant capacity (TEAC) per gram.

Determination of Total Phenolic Content of Mushrooms

An adapted Folin–Ciocalteu method was used to determine the total phenolic content of the mushrooms [57]. In summary, 30 μL of the mushroom extracts were added on microplate wells containing 150 μL of Folin–Ciocalteu (Sigma-Aldrich, Darmstadt, Germany) solution at 10% ($v v^{-1}$) and 120 μL of sodium carbonate (Synth, Diadema, Brazil) 4% ($m v^{-1}$). The microplate was kept in the dark for 2 h and the absorbance was measured at 750 nm (Multiskan GO[®]—Thermo Scientific). A standard curve was performed using gallic acid (Sigma-Aldrich, Darmstadt, Germany) and the

results were expressed as mg of gallic acid equivalents per 100 g of sample ($\text{mg GAE } 100 \text{ g}^{-1}$).

Results and Discussion

Composition of Raw Material

The composition of CB and BSG vary considerably according to the variety cultivated, soil, the method, and period of cultivation, in addition to the processing to which they are subjected [58–63]. The immersion of raw materials in a hydrated lime solution ($2\% m v^{-1}$) raised the pH of the CB (Table 1), indicating that this material absorbed elements from the alkaline solution. As expected, the ash and calcium content of both treated raw materials were higher. On the other hand, the alkaline treatment reduced their crude fiber content (Table 1), which could be attributed to the break of ester and ether bonds between some fibers, such as hemicellulose and lignin. This increases their solubility and, consequently, reduces the fiber content of lignocellulosic residues [64, 65].

Percentage Yield of Cultivated Mushrooms

There were no mushrooms grown in the substrates of treatments T1, T2, and T3, with or without Se, especially due to the development of contaminants. The immersion of the

Table 1 pH, proximate composition in dry basis (% DB), and minerals of by-products before and after immersion in hydrated lime

Substrates						
Parameters	CB			BSG		
	Before	After	Difference	Before	After	Difference
pH	4.5 \pm 0.27	11.1 \pm 0.07	6.6 \pm 0.32 *	5.5 \pm 0.09	6.0 \pm 0.14	0.5 \pm 0.14 ns
Composition						
Crude protein	1.36 \pm 0.20	1.37 \pm 0.06	0.01 \pm 0.26 ns	20.04 \pm 0.20	19.81 \pm 0.39	-0.23 \pm 0.19 ns
Fat	0.43 \pm 0.01	0.43 \pm 0.01	0.00 \pm 0.00 ns	7.28 \pm 0.12	3.11 \pm 0.03	-4.17 \pm 0.15 *
Crude fiber	54.17 \pm 0.50	49.70 \pm 0.13	-4.47 \pm 0.38 *	25.46 \pm 0.12	20.61 \pm 0.79	-4.85 \pm 0.66 *
Ash	0.40 \pm 0.00	10.11 \pm 0.30	9.71 \pm 0.30 *	4.46 \pm 0.01	5.63 \pm 0.12	1.17 \pm 0.13 ns
NFE	43.64 \pm 0.30	38.40 \pm 0.12	-5.24 \pm 0.42 *	42.76 \pm 0.43	50.84 \pm 1.27	8.08 \pm 0.84 *
Minerals						
Se ($\mu\text{g g}^{-1}$)	Nd	0.132 \pm 0.019	-	Nd	0.125 \pm 0.017	-
N (mg g^{-1})	2.18 \pm 0.32	2.19 \pm 0.10	0.01 \pm 0.41 ns	34.38 \pm 0.34	33.97 \pm 0.67	-0.40 \pm 0.33 ns
P (mg g^{-1})	0.45 \pm 0.07	0.80 \pm 0.14	0.35 \pm 0.07 ns	5.35 \pm 0.07	4.60 \pm 0.28	-0.75 \pm 0.21 ns
K (mg g^{-1})	0.80 \pm 0.00	1.80 \pm 0.28	1.00 \pm 0.28 *	1.00 \pm 0.28	0.80 \pm 0.00	-0.20 \pm 0.28 ns
Ca (mg g^{-1})	1.45 \pm 0.07	26.70 \pm 1.13	25.25 \pm 1.06 *	1.80 \pm 0.00	10.75 \pm 0.07	8.95 \pm 0.07 *
Mg (mg g^{-1})	0.10 \pm 0.55	0.55 \pm 0.07	0.45 \pm 0.07 *	2.05 \pm 0.07	1.40 \pm 0.00	-0.65 \pm 0.07 *
Na (mg g^{-1})	0.03 \pm 0.00	0.09 \pm 0.01	0.06 \pm 0.01 *	0.04 \pm 0.01	0.07 \pm 0.00	0.04 \pm 0.01 ns
Fe (mg g^{-1})	0.07 \pm 0.01	0.14 \pm 0.00	0.08 \pm 0.01 *	0.15 \pm 0.00	0.14 \pm 0.00	0.00 \pm 0.01 ns

CB=sugarcane bagasse. BSG=brewer's spent grain. NFE=Nitrogen-free extract. nd=non-determined. Data relative to each by-product before and after immersion in hydrated lime solution were compared using T-test: ^{ns}non-significant; * $p < 0.05$

substrates in the hydrated lime solution should increase the pH of the substrate so that contaminants, such as larvae, flies, and *Coprinus* spp., do not develop [66–68]. However, the increase in the pH of BSG (from 5.5 to 6.0—Table 1) was not enough, making the substrates composed mainly of this by-product (T1, T2, and T3) favorable environments for the development of the previously present contaminants. Thus, the treatment with hydrated lime, under the conditions used in this study, is not recommended for the preparation of this raw material for mushroom production, when used in larger quantities.

Although this method is not the most appropriate for some by-products, it is less expensive than sterilization. It only requires buying the reagent $\text{Ca}(\text{OH})_2$ and equipment to remove excess solution, such as a filter or centrifuge. Sterilization is mainly performed using an autoclave, with high electricity costs [13].

Thus, only the substrates consisting of CB:BSG at 75:25 (T4) and at 100:0 (T5) were suitable for the production of mushrooms using an alkaline treatment, whose appearances

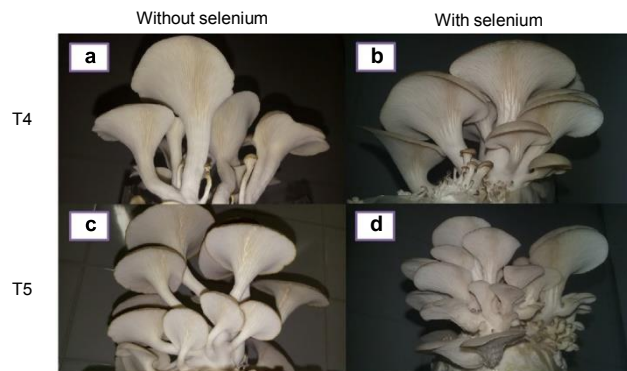


Fig. 1 *Pleurotus ostreatus* mushrooms grown on substrate based on CB:BSG at 75:25 (T4) without selenium (a) and with selenium (b) and grown on pure CB (T5) without selenium (c) and with selenium (d). CB sugarcane bagasse and BSG brewer's spent grain

Table 2 Production yield of *Pleurotus ostreatus* mushrooms grown in substrates based on different proportions of CB and BSG, enriched or not with Se (sodium selenite) at 25 mg kg^{-1}

Yield	Substrates	Without Se	With Se
Days after inoculation (d)	T4	36.00 ± 0.00 a B	39.40 ± 1.82 a A
	T5	33.40 ± 0.89 a B	39.40 ± 5.08 a A
Fresh weight (g)	T4	40.40 ± 6.11 b A	45.20 ± 9.18 b A
	T5	59.00 ± 12.88 a A	57.40 ± 12.42 a A
Dry weight (g per bag)	T4	6.82 ± 1.03 a A	6.99 ± 0.90 a A
	T5	7.54 ± 1.95 a A	7.19 ± 1.66 a A
BE (g 100 g^{-1})	T4	33.79 ± 5.11 b A	37.80 ± 7.67 b A
	T5	52.31 ± 11.42 a A	50.89 ± 11.01 a A

BE = Biological efficiency. T4 = substrate composed of CB:BSG (75:25). T5 = substrate composed of pure CB. CB: sugarcane bagasse and BSG: brewer's spent grain. For each parameter analyzed, averages with different letters in the columns (lower case) or lines (upper case) differ significantly by Tukey test ($p < 0.05$)

were not negatively affected by the presence of Se (Fig. 1). However, the addition of Na_2SeO_3 to the substrates delayed the production of the mushrooms (Table 2), as previously observed in *P. ostreatus*, *P. djamor*, and *P. cornucopiae* cultivation [17, 24, 38]. The delay in mushroom production is probably due to the need for the fungus to adapt to the substrate enriched with Na_2SeO_3 since this compound is a highly reactive inorganic form of Se and can present some toxicity [27]. Despite the delay, the presence of Na_2SeO_3 did not affect the fresh weight, dry weight, or BE of the mushrooms. In general, using pure CB (T5) provided a higher yield for mushroom production of *P. ostreatus* (Table 2).

Among these parameters, the BE is the most used because it considers the fresh weight of the mushrooms with the dry weight of the substrates, whose moisture content could influence the BE [69]. The BE of Se-enriched mushrooms varies considerably depending on the fungal isolate, the substrate, and the form and concentration of Se (Table 3). In this work, the BE of the *P. ostreatus* mushroom was higher (33.79–52.31%) than that observed for the same species cultivated on coffee husks (25.26%) enriched with Se at 25.4 mg kg^{-1} (Na_2SeO_3) [24], but lower than the BE obtained when it was grown on wheat straw from seleniferous soil (27 mg kg^{-1} of Se) [68]. Thus, the by-products can provide different BE for the same fungi species due to differences in substrate composition.

Proximate Composition of Cultivated Mushrooms

The nitrogen-free extract (NFE) of the mushrooms varied from 57.51 to 65.20%, similarly to that found in *P. ostreatus* and *P. pulmonarius* (54.36–58.10%) cultivated in substrates enriched or not with Na_2SeO_3 [40]. Generally, this is the main component of *Pleurotus* spp., in dry basis [70]. High contents of protein (17.07–23.50%), fiber (11.13–13.32%) and minerals (ash) (4.46–7.21%), and low fat (0.56–1.42%) were found (Table 4). Therefore, these mushrooms can be considered excellent sources of nutrients for low-calorie

Table 3 Selenium added or naturally present in different substrates, the content of selenium in the mushrooms and BE of different species of the mushrooms

Mushroom	By-products	Se form	Se concentration ($\mu\text{g g}^{-1}$)		BE (g 100 g ⁻¹)	Reference
			Substrate	Mushroom		
<i>C. indica</i>	Wheat straw	Na ₂ SeO ₃	10	8.28	64.53	[37]
<i>P. eryngii</i>	Beech sawdust: wheat straw (1:1)	Na ₂ SeO ₃	50	13.6	12.28	[73]
<i>P. eryngii</i>	Mainly cottonseed hulls	Na ₂ SeO ₃	14.4	9.3	71.9	[82]
<i>P. eryngii</i>	Mainly cottonseed hulls	Na ₂ SeO ₃	8.1	4.6	65.1	[82]
<i>P. ostreatus</i>	Coffee husks	Na ₂ SeO ₃	25.4	310.21	25.26	[24]
<i>P. ostreatus</i>	Coffee husks	Na ₂ SeO ₃	12.7	175.42	35.56	[24]
<i>P. ostreatus</i>	CB	Na ₂ SeO ₃	25	119.3	40	[17]
<i>P. ostreatus</i>	CB	Na ₂ SeO ₃	12.5	86.45	45.71	[17]
<i>P. ostreatus</i>	Red sorghum straw	Na ₂ SeO ₄	17.5	15.14	93.33	[78]
<i>P. ostreatus</i>	Red sorghum straw	Na ₂ SeO ₄	5.8	2.62	89.09	[78]
<i>P. ostreatus</i>	Wheat straw	Substrate from Se-rich soil	40.5	182.2	26.07	[35]
<i>P. ostreatus</i>	Wheat straw	Substrate from Se-rich soil	27	405	78.34	[74]
<i>P. citrinopileatus</i>	Wheat straw	Substrate from Se-rich soil	27	306	74.8	[74]
<i>P. florida</i>	Wheat straw	Substrate from Se-rich soil	40.5	184.3	64.5	[35]
<i>P. sajor caju</i>	Wheat straw	Substrate from Se-rich soil	40.5	190.5	30.46	[35]
<i>P. sajor-caju</i>	Wheat straw	Substrate from Se-rich soil	27	399	80	[74]
<i>V. volvacea</i>	Paddy straw	Substrate from Se-rich soil	29.7	231	19.32	[74]

BE=Biological efficiency. $\mu\text{g g}^{-1} = \text{mg kg}^{-1}$

diets, especially for vegans, who need alternative sources of protein. In general, the pure CB (T5) produced mushrooms with higher fat and fiber content, while the presence of BSG increased the ash content and, when not enriched with Se, the protein content of the mushrooms. Therefore, this by-product should be tested in proportions lower than 25% for mushroom production, under the same conditions used in our work, because it is a by-product present in many countries [2] and can be a source of nitrogen (Table 1) for mushrooms. The addition of Na₂SeO₃ also leads to the production of mushrooms with higher fiber and ash contents.

The influence of substrate composition and Se addition on the centesimal composition of mushrooms has been previously reported. Rice straw, for example, generates *P. ostreatus* mushrooms with 13.00% of crude protein, 6.13% of ash, and 6.32% of fat [71], while softwood sawdust (*Daniellia oliveri*) generated mushrooms with 17.68% and 10.66% of crude protein and crude fiber, respectively, and in hardwood sawdust (*Anogeissus leiocarpus*) the mushrooms presented 26.67% and 11.05% of these components [72]. The addition of Se (Na₂SeO₃) to wheat straw generated *C. indica*

Table 4 Proximate composition (% dry basis) of *Pleurotus ostreatus* mushrooms cultivated in substrates with or without selenium (sodium selenite) at 25 mg kg⁻¹

Composition (%)	Substrates	Without Se	With Se
Crude protein	T4	23.50±0.69 a A	17.69±0.81 a B
	T5	17.07±0.36 b A	18.09±0.06 a A
Fat	T4	0.92±0.06 b A	0.56±0.01 b B
	T5	1.18±0.08 a B	1.42±0.11 a A
Crude fiber	T4	11.13±0.61 b A	11.68±0.37 b A
	T5	12.08±0.18 a A	13.32±1.36 a A
Ash	T4	6.94±0.50 a A	7.21±0.01 a A
	T5	4.46±0.00 b B	6.76±0.18 b A
NFE	T4	57.51±0.65 b B	62.87±0.44 a A
	T5	65.20±0.62 a A	60.41±1.23 b B

T4 = substrate composed of CB:BSG (75:25). T5 = substrate composed of pure CB. CB: sugarcane bagasse and BSG: brewer's spent grain. NFE = Nitrogen-free extract. For each parameter analyzed, averages with different letters in the columns (lower case) or lines (upper case) differ significantly by Tukey test ($p < 0.05$)

mushrooms with higher protein content (20.14%) than mushrooms grown on a non-enriched substrate (14.33%) [37]. Similarly, when *P. pulmonarius* was cultivated on corncob, the addition of Se (Na_2SeO_3) increased the protein (from 10.31 to 16.35%) and ash (from 6.20 to 8.21%) contents of the mushrooms and reduced their fiber content (from 19.70 to 11.82%) [40].

Mineral Composition of Cultivated Mushrooms

The use of Na_2SeO_3 increased the Se content in *P. ostreatus* mushrooms, although, in the substrate containing pure CB (T5), the mushrooms presented more capacity to absorb the mineral (Table 5), as well as Na and Fe, considering that the concentrations of these minerals were similar in CB and BSG. Sodium absorption was positively influenced by the presence of Se in substrate T4, as well as N and K absorption in pure CB (T5). On the other hand, the P and Mg concentrations of the mushrooms were proportional to their concentrations in the respective substrates, so they were lower

in mushrooms cultivated in pure CB. The substrates had extremely high Ca concentrations due to the disinfection treatment, so all mushrooms absorbed this mineral in similar amounts (0.30 mg g^{-1}), possibly their maximum absorption capacity. The Se and K concentrations in the mushrooms were correlated positively ($p < 0.1$).

Apparently, besides the addition of Na_2SeO_3 , the composition of the substrate in which the mushroom was grown influenced the mineral absorption and its incorporation by the mushrooms. The Se concentrations found in our work (127.03 and $179.07 \text{ } \mu\text{g g}^{-1}$) were higher than those found in *P. eryngii* ($13.6 \text{ } \mu\text{g g}^{-1}$) [73] and *C. indica* ($8.28 \text{ } \mu\text{g g}^{-1}$) [37] grown on substrates containing wheat straw enriched with 50 and 10 mg kg^{-1} of Se (Na_2SeO_3), respectively. Se contents ($0.4\text{--}15 \text{ } \mu\text{g g}^{-1}$) found in *C. indica* mushrooms grown on various substrates (wheat straw and leaves of *Bauhinia variegata*, *Syzygium cumini*, and *Syzygium cumini*) were correlated and proportional to Ca ($0.06\text{--}0.18 \text{ mg g}^{-1}$), Mg ($2.54\text{--}4.27 \text{ mg g}^{-1}$), and Na ($0.13\text{--}0.42 \text{ mg g}^{-1}$) contents [75]. In *P. ostreatus* grown on a substrate composed

Table 5 Mineral composition of *Pleurotus ostreatus* mushrooms cultivated in substrates with or without selenium (sodium selenite) at 25 mg kg^{-1} and Pearson's correlation coefficients (r) between Se concentrations in mushrooms and others mineral content

Minerals	Substrates	Without Se	With Se
Se ($\mu\text{g g}^{-1}$)	T4	0.16 ± 0.12	127.03 ± 2.05
	T5	0.09 ± 0.02	179.07 ± 12.79
N (mg g^{-1})	T4	5.37 ± 0.16	4.04 ± 0.18
	T5	3.90 ± 0.08	4.13 ± 0.01
P (mg g^{-1})	T4	12.10 ± 0.57	12.55 ± 0.64
	T5	7.05 ± 0.64	7.20 ± 0.14
K (mg g^{-1})	T4	21.60 ± 0.00	21.40 ± 0.28
	T5	18.60 ± 1.98	23.00 ± 0.28
Ca (mg g^{-1})	T4	0.30 ± 0.00	0.30 ± 0.14
	T5	0.30 ± 0.00	0.30 ± 0.14
Mg (mg g^{-1})	T4	1.35 ± 0.07	1.45 ± 0.07
	T5	1.15 ± 0.07	1.15 ± 0.07
Na (mg g^{-1})	T4	0.14 ± 0.00	0.19 ± 0.01
	T5	0.27 ± 0.01	0.28 ± 0.00
Fe (mg g^{-1})	T4	0.07 ± 0.01	0.07 ± 0.01
	T5	0.10 ± 0.01	0.09 ± 0.00
Pearson's correlation			
	Se		
N		-0.44^{ns}	
P		-0.09^{ns}	
K		0.65^*	
Ca		0.01^{ns}	
Mg		0.02^{ns}	
Na		0.37^{ns}	
Fe		0.12^{ns}	

T4=substrate composed of CB:BSG (75:25). T5=substrate composed of pure CB. CB: sugarcane bagasse and BSG: brewer's spent grain. Pearson's correlation: ^{ns}non-significant; * $p < 0.1$

mainly of CB, the addition of Na_2SeO_3 reduced the Ca absorption capacity (from 0.034 to 0.025 mg g^{-1}) [25], but when cultivated on corncob, the addition of this salt increased the absorption capacity of this mineral (from 0.018 to 0.209 mg g^{-1}) [40]. The use of Na_2SeO_3 in beech sawdust:wheat straw (1:1) increased the Mg (from 1.17 to 2.35 mg g^{-1}) and Fe (from 0.039 to 0.051 mg g^{-1}) contents in *P. eryngii* mushrooms [73]. However, in *P. ostreatus* mushrooms grown on corncob, this salt increased the Mg (from 0.026 to 0.038 mg g^{-1}) and reduced the Fe (from 0.022 to 0.007 mg g^{-1}) contents [40].

Daily consumption of only 0.31 g of Se-enriched mushrooms produced in pure CB (T5) could meet the daily demand for Se, which is 55 μg per day. It could also help meet the daily demands for other minerals, such as P and K, which are 700 and 4,700 mg per day, respectively, for healthy adults [76].

Antioxidant Activity and Phenolic Content of Mushrooms

The substrate composed of pure CB (T5) generated mushrooms with higher antioxidant activity measured by DPPH, but not by ABTS (Table 6). The higher total phenolic content in the mushrooms was observed when mushrooms were cultivated in Se-enriched substrate CB:BSG at 75:25 (T4), but it did not reflect a proportional increase in the antioxidant activity (measured by DPPH). The phenolic content was correlated to the antioxidant activity measured by ABTS, but the Se content in the mushrooms was not correlated to

their antioxidant activity (DPPH and ABTS) nor the total phenolic content. The methods of extraction and evaluation of antioxidant activity and phenolic content of mushrooms differ considerably [35, 77–79], so there needs to be caution when comparing results from different works.

In contrast with the findings of our work, the addition of Se proportionally increased the total phenolic content and antioxidant activity in mushrooms [77, 79, 80]. Gąsecka et al. [77], for example, observed that the increase in the Se content in mushrooms was proportional to the increase in the phenolic content (from 8.7 to 12.5 mg g^{-1}), and the antioxidant activity measured by DPPH (% FRS) (from 47.1 to 51.1%) and ABTS (% FRS) (from 54.2 to 63.1%) in *Pholiota Nameko* mushrooms. Se-enriched *Ganoderma lucidum* mushrooms also presented a proportional increase in phenolic content (from 28.12 to 40.31 mg g^{-1}) and antioxidant activity measured by DPPH (% FRS) (from 60.93 to 86.99%), in comparison with non-enriched mushrooms [80]. Possibly, the synthesis of phenolic and consequently increased antioxidant activity in those mushrooms result from the stress caused by growing on Se-rich substrate [77]. However, considering that the nature of the substrate also influences the mushroom composition, the discrepancy among the results of different studies could also be attributed to the diversity of compounds with antioxidant capacity present in the raw material [81]. As observed here, adding Se to the substrate can affect other mushroom compounds, besides phenolics [80], such as proteins, fibers, lipids, and minerals (Tables 4 and 5) and this may have also influenced the antioxidant response.

Table 6 Antioxidant activity by DPPH, ABTS and total phenolic content of *Pleurotus ostreatus* mushrooms cultivated in substrates with or without sodium selenite (Se) at 25 mg kg^{-1} and Pearson's correla-

tion coefficients between DPPH, ABTS, total phenolic content and Se concentrations

Analysis	Substrates	Without Se	With Se	Positive control (BHT)
DPPH (% FRS)	T4	32.79 ± 0.59 b A	37.10 ± 3.87 b A	95.21 ± 0.14
	T5	42.49 ± 1.26 a A	44.67 ± 2.26 a A	
ABTS (μM TEAC g^{-1})	T4	25.42 ± 6.99 a A	25.38 ± 5.70 a A	232.67 ± 28.33
	T5	26.24 ± 4.32 a A	20.19 ± 3.15 a A	
Phenolics (mg EAG 100 g^{-1})	T4	708.66 ± 117.48 a A	754.96 ± 30.13 a A	–
	T5	664.34 ± 178.10 a A	458.78 ± 48.44 b A	
Pearson's correlation				
		DPPH	ABTS	Phenolics
DPPH		1		
ABTS		– 0.16 ^{ns}	1	
Phenolics		– 0.55*	0.79**	1
Se		0.45 ^{ns}	– 0.38 ^{ns}	– 0.43 ^{ns}

T4 = substrate composed of CB:BSG (75:25). T5 = substrate composed of pure CB. CB: sugarcane bagasse and BSG: brewer's spent grain. For each parameter analyzed, averages with different letters in the columns (lower case) or lines (upper case) differ significantly by the Tukey test ($p < 0.05$). Pearson's correlation: ^{ns}non-significant; * $p < 0.1$; ** $p < 0.05$

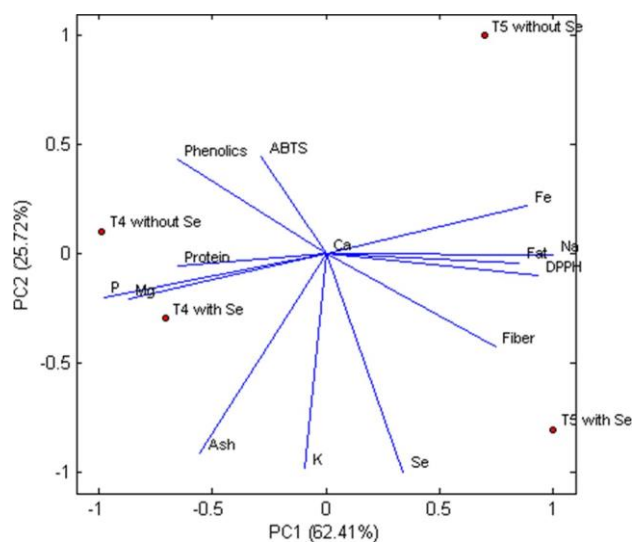


Fig. 2 Principal component analysis of characteristics of *P. ostreatus* grown in the presence of Se. T4 = substrate composed of CB:BSG (75:25). T5 = substrate composed of pure CB. CB sugarcane bagasse and BSG brewer's spent grain

Effect of Selenium Enrichment on the Mushrooms

The addition of Na_2SeO_3 to the substrates affected the mushroom characteristics differently, depending on the substrate used for mushroom cultivation. Thus, a principal component analysis (PCA) was performed to identify the mushroom characteristics that were most influenced by the addition of Na_2SeO_3 to the substrate (Fig. 2). The principal component 1 (PC1) explains 62.41% of the variability for the evaluated parameters, while the principal component 2 (PC2) explains 25.72%. Together, PC1 and PC2 explain 88.13% of the variability.

Se-enrichment influenced mainly the fiber and Se contents of the *P. ostreatus* mushrooms cultivated on T5 (pure CB) (Fig. 2). The contents of P, Mg, and protein were closer to the substrate T4 (CB:BSG at 75:25), independent of the addition of Se. In our work, Se only correlated more with K (Table 5 and Fig. 2), in contrast to *C. indica* mushrooms cultivated in wheat straw, in which Se was more correlated to Ca, Na, and protein [37, 75], and also in contrast to *P. eryngii* mushrooms cultivated in beech sawdust:wheat straw (1:1), in which Se was more correlated with Mg [73]. This may indicate that the fungi species, the use of Se, and the substrate composition differently influence the absorption and the ability of mushrooms to convert the elements to other substances. For this reason, new studies verifying the influence of using Se in the substrate on mushroom characteristics are necessary to investigate whether there is a pattern according to the mushroom species and the substrate used.

Conclusions

The production of Se-enriched *P. ostreatus* mushrooms can use sugarcane bagasse (CB) at 75% with BSG at 25%, but using pure CB has led to increased yield, antioxidant activity, and Se content of Se-enriched *P. ostreatus* mushrooms. Thus, CB treated with hydrated lime solution ($\text{Ca}(\text{OH})_2$) ($2\% \text{ m v}^{-1}$) was considered the best suitable substrate. This strategy can be used as an inexpensive and efficient way to produce mushrooms. On the other hand, when cultivated on CB combined with BSG the mushrooms presented higher protein content, in the absence of Se. For this reason, we suggest that further work be done to test the use of proportions lower than 25% of BSG in combination with CB for the production of Se-enriched mushrooms under the same conditions used in our work. The Se-enriched mushrooms also can be used in future experiments with animals.

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Declarations

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References

1. FAOSTAT: Crops. <http://www.fao.org/faostat/en/#data/QC> (2019). Accessed 14 March 2021

2. Kirin Holdings Company: Kirin Beer University Report Global Beer Production by Country in 2018. https://www.kirinholdings.co.jp/english/news/2019/1003_01.html (2018). Accessed 14 March 2021
3. Christofolletti, C.A., Moreira-de-Sousa, C., Souza, R.B. et al.: O emprego de diferentes resíduos utilizados como fertilizantes na cultura de cana-de-açúcar. In: Fontanetti, C.S., Bueno, O.C. (eds.). *Cana-de-açúcar e seus impactos: uma visão acadêmica*, pp. 63–86. Canal 6 editora, Bauru (2017)
4. Reinold, M.R.: *Manual Prático de Cervejaria*. Aden Editora e Comunicacoes Ltda, São Paulo (1997)
5. Eggleston, G., Lima, I.: Sustainability issues and opportunities in the sugar and sugar-bioproduct industries. *Sustainability* **7**, 12209–12235 (2015)
6. Lynch, K.M., Steffen, E.J., Arendt, E.K.: Brewers' spent grain: a review with an emphasis on food and health. *J. Inst. Brew.* **122**, 553–568 (2016)
7. González-García, S., Morales, P.C., Gullón, B.: Estimating the environmental impacts of a brewery waste-based biorefinery: Bioethanol and xylooligosaccharides joint production case study. *Ind. Crops Prod.* **123**, 331–340 (2018)
8. Bianco, A., Budroni, M., Zara, S., Mannazzu, I., et al.: The role of microorganisms on biotransformation of brewers' spent grain. *Appl. Microbiol. Biotechnol.* **104**, 8661–8678 (2020)
9. Mussatto, S.I.: Brewer's spent grain: a valuable feedstock for industrial applications. *J. Sci. Food Agric.* **94**, 1264–1275 (2014)
10. Thakur, V.K., Thakur, M.K., Raghavan, P.: Progress in green polymer composites from lignin for multifunctional applications: a review. *ACS Sustain. Chem. Eng.* **2**, 1072–1092 (2014)
11. Karp, S.J., Faraco, V., Amore, A., et al.: Statistical optimization of lacase production and delignification of sugarcane bagasse by *Pleurotus ostreatus* in solid-state fermentation. *Biomed. Res. Int.* **2015**, 1–8 (2015)
12. Rai, R.D.: Production of edible Fungi. In: Arora, D.K. (ed.) *Fungal Biotechnology in Agricultural, Food, and Environmental Applications*, vol. 21, pp. 233–246. Marcel Dekker, New York (2004)
13. Nunes, M.D., Da Silva, M.C.S., Schram, J.G.S., et al.: *Pleurotus ostreatus*, mushrooms production using quick and cheap methods and the challenges to the use of coffee husk as substrate. *Afr. J. Microbiol. Res.* **11**, 1252–1258 (2017)
14. Gowda, N.N., Manvi, D.: Agro-residues disinfection methods for mushroom cultivation. *Agric. Rev.* **40**, 93–103 (2019)
15. Contreras, E.P., Sokolov, M., Mejía, G.: Soaking the substrate in alkaline water as a pretreatment for the cultivation of *Pleurotus ostreatus*. *J. Hortic. Sci. Biotechnol.* **79**, 234–240 (2004)
16. Avendaño-Hernandez, R.J., Sánchez, J.E.: Self-pasteurization substrate for growing oyster mushrooms (*Pleurotus* spp.). *Afr. J. Microbiol. Res.* **7**, 220–226 (2013)
17. Sereño, P.A.Z.: Enriquecimento, distribuição e especiação de selênio em cogumelos de *Pleurotus* spp. (Master's dissertation, Universidade Federal de Viçosa) (2016) <https://www.locus.ufv.br/bitstream/123456789/10062/1/texto%20completo.pdf>. Accessed 15 March 2021
18. León-Monzón, J.H., Sánchez, J.E., Nahed-Toral, J.: El cultivo de *Pleurotus ostreatus* en los Altos de Chiapas, México. *Rev. Mex. Micol.* **18**, 31–38 (2004)
19. Soto, B.: Fermentation in food processing: microbiology and biotechnology. In: Soto, B. (ed.) *Fermentation processes*, pp. 173–248. ED Tech Press, Waltham Abbey (2019)
20. Chang, S.T., Miles, P.G.: *Mushrooms: Cultivation, Nutritional Value Medicinal Effect and Environmental Impact*, 2nd edn. CRC Press, Boca Raton (2004)
21. Husan, A., Jawaid, M.: *Nanomaterials for Agriculture and Forestry Applications*. Elsevier, Amsterdam (2020)
22. Ooi, V.E.C.: Antitumor and immunomodulatory activities of mushroom polysaccharides. In: Cheung, P.C.K. (ed.) *Mushrooms as Functional Foods*, 1st edn., pp. 147–198. Wiley, Hoboken (2008)
23. Oyetayo, V.O., Ariyo, O.O.: Micro and macronutrient properties of *Pleurotus ostreatus* (Jacq:Fries) cultivated on different wood substrates. *Jordan J. Biol. Sci.* **6**, 223–226 (2013)
24. Da Silva, M.C.S., Naozuka, J., Luz, J.M.R., et al.: Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. *Food Chem.* **131**, 558–563 (2012)
25. Oliveira, A.P., Leme, F.O., Nomura, C.S., et al.: Elemental imaging by Laser induced breakdown spectroscopy to evaluate selenium enrichment effects in edible mushrooms. *Nature* **9**, 1–10 (2019)
26. Maseko, T., Howell, K., Dunshea, F.R., et al.: Selenium-enriched *Agaricus bisporus* increases expression and activity of glutathione peroxidase-1 and expression of glutathione peroxidase-2 in rat colon. *Food Chem.* **146**, 327–333 (2014)
27. Prauchner, C.A.: *A Importância do Selênio para a Agropecuária e Saúde Humana*. UFSM, Santa Maria (2014)
28. Reilly, C.: *Selenium in Food and Health*, 2nd edn. Springer, Brisbane (2006)
29. Nève, J.: Selenium in nutrition and therapeutics. In: Bittar, E.E., Bittar, N. (eds.) *Principles of Medical Biology*, vol. 8, pp. 985–994. Elsevier, Amsterdam (1997)
30. Zhou, F., Dinh, Q.T., Yang, W., et al.: Assessment of speciation and in vitro bioaccessibility of selenium in Selenium-enriched *Pleurotus ostreatus* and potential health risks. *Ecotoxicol. Environ. Saf.* **185**, 1–9 (2019)
31. Niedzielski, P., Mleczek, M., Siwulski, M., et al.: Efficacy of supplementation of selected medicinal mushrooms with inorganic selenium salts. *J. Environ. Sci. Health B* **49**, 929–937 (2014)
32. Yoshida, M., Sugihara, S., Inoue, Y., et al.: Composition of chemical species of selenium contained in selenium-enriched Shiitake mushroom and vegetables determined by high performance liquid chromatography with inductively coupled plasma mass spectrometry. *J. Nutr. Sci. Vitaminol.* **51**, 194–199 (2005)
33. Milovanović, I., Brčeski, I., Stajić, M., et al.: Potential of *Pleurotus ostreatus* mycelium for selenium absorption. *Sci. World J.* **2014**, 1–8 (2014)
34. Rzymiski, P., Mleczek, M., Niedzielski, P., et al.: Cultivation of *Agaricus bisporus* enriched with selenium, zinc and copper. *J. Sci. Food Agric.* **97**, 923–928 (2016)
35. Kaur, G., Kalia, A., Sodhi, H.S.: Selenium biofortification of *Pleurotus* species and its effect on yield, phytochemical profiles, and protein chemistry of fruiting bodies. *J. Food Biochem.* **42**, 1–7 (2017)
36. Golian, M., Hegedúsová, A., Trochcová, M., et al.: The effect of sodium selenate application on growth of the fruiting bodies in the first flush of mushroom *Pleurotus ostreatus* (Jacq.) P. Kumm. *Acta Hort. Regiotechnicae* **2018**, 25–29 (2018)
37. Rathore, H., Sharma, A., Prasad, S., et al.: Selenium bioaccumulation and associated nutraceutical properties in *Calocybe indica* mushroom cultivated on Se-enriched wheat straw. *J. Biosci. Bioeng.* **126**, 1–6 (2018)
38. Da Silva, M.C.S., Luz, J.M.R., Paiva, A.P.S., et al.: Growth and tolerance of *Pleurotus ostreatus* at different selenium forms. *J. Agric. Sci.* **11**, 151–158 (2019)
39. Assunção, L.S., Da Silva, M.C.S., Fernandez, M.G., et al.: Speciation of selenium in *Pleurotus ostreatus* and *Lentinula edodes* mushrooms. *J. Biotechnol. Lett.* **5**, 79–86 (2014)
40. Fasantí, O.F., Ogidí, C.O., Oyetayo, V.O.: Nutrient contents and antioxidant properties of *Pleurotus* spp. cultivated on substrate fortified with selenium. *Curr. Res. Environ. Appl. Mycol.* **9**, 66–76 (2019)

41. Ogidi, C.O., Nunes, M.D., Da Silva, M.C.S., et al.: Growth rate and selenium bioaccumulation in *Pleurotus* species cultivated on signal grass, *Urochloa decumbens* (Stapf) R. D. Webster. *Curr. Res. Nutr. Food Sci.* **5**, 137–143 (2017)
42. Kasuya, M.C.M., Luz, J.M.R., Nunes, M.D., et al.: Production of selenium-enriched mushrooms in coffee husks and use of this colonized residue. In: Preedy, V.R. (ed.) *Coffee in Health and Disease Prevention*, pp. 301–309. Elsevier, Amsterdam (2015)
43. Association of Official Analytical Chemists – AOAC: *Official Methods of Analysis of AOAC International*, 17th edn. AOAC International, Gaithersburg (2000)
44. Jones, D.B.: Factors for converting percentages of nitrogen in foods and feeds into percentages of protein. US Department of Agriculture - circ. 183. Washington, D.C. http://toolbox.foodcomp.info/References/Protein/Jones_1941%20nitrogen-protein%20conversion%20cir183.pdf (1941). Accessed 18 March 2021
45. Detmann, E., Souza, M.A., Valadares Filho, S.C. et al.: *Métodos para Análise de Alimentos - INCT - Ciência Animal*. Suprema, Visconde do Rio Branco (2012)
46. Silva, D.J., Queiroz, A.C.: *Análise de Alimentos (Métodos Químicos e Biológicos)*. 3.ed. UFV, Viçosa (2006)
47. Oliveira, A.P., Naozuka, J.: Preliminary results on the feasibility of producing selenium-enriched pink (*Pleurotus djamor*) and white (*Pleurotus ostreatus*) oyster mushrooms: Bioaccumulation, bioaccessibility, and Se-proteins distribution. *Microchim. J.* **145**, 1143–1150 (2019)
48. Frank, A.: Automated wet ashing and multi-metal determination in biological materials by atomic-absorption spectrometry. *Fresenius' Z. Anal. Chem.* **279**, 101–102 (1976)
49. Braga, J.M., Defelipo, B.V.: Determinação espectrofotométrica de fósforo em extratos de solo e material vegetal. *Rev. Ceres* **21**, 73–85 (1974)
50. Pearson, K.: LIII. On lines and planes of closest fit to systems of points in space. *Lond. Edinb. Dublin Philos. Mag. J. Sci.* **2**, 559–572 (1901)
51. Nunes, C.A., Freitas, M.P., Pinheiro, A.C.M., et al.: Chemoface: a novel free user-friendly interface for chemometrics. *J. Braz. Chem. Soc.* **23**, 2003–2010 (2012)
52. Wang, D., Sakoda, A., Suzuki, M.: Biological efficiency and nutritional value of *Pleurotus ostreatus* cultivated on spent beer grain. *Biores. Technol.* **78**, 293–300 (2001)
53. Breene, W.M.: Nutritional and medicinal value of specialty mushrooms. *J. Food Prot.* **53**, 883–894 (1990)
54. Kähkönen, M.P., Hopia, A.I., Heikki, J.V., et al.: Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* **47**, 3954–3962 (1999)
55. Brand-Williams, W., Cuvelier, M.E., Berset, C.: Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci. Technol.* **28**, 25–30 (1995)
56. Nenadis, N., Wang, L.F., Tsimidou, M., et al.: Estimation of scavenging activity of phenolic compounds using the ABTS⁺ assay. *J. Agric. Food Chem.* **52**, 4669–4674 (2004)
57. Waterhouse, A.L.: Wine phenolics. *Ann. N.Y. Acad. Sci.* **957**, 21–36 (2002)
58. Ajanaku, K.O., Dawodu, F.A., Ajanaku, C.O., Nwinyi, O.C.: Functional and nutritional properties of spent grain enhanced cookies. *Am. J. Food Technol.* **6**, 763–771 (2011)
59. Carvalho, F., Esteves, M., Parajó, J., Pereira, H., Girio, F.: Production of oligosaccharides by autohydrolysis of brewery's spent grain. *Biores. Technol.* **91**, 93–100 (2004)
60. Coradi, G.V., Visitação, V.L., Lima, E.A., Saito, L.Y.T., Palmieri, D.A., Takita, M.A., Neto, P.O., Lima, V.M.G.: Comparing submerged and solid-state fermentation of agro-industrial residues for the production and characterization of lipase by *Trichoderma harzianum*. *Ann. Microbiol.* **63**, 533–540 (2013)
61. Siqueira, F.G., Siqueira, A.G., Siqueira, E.G., Carvalho, M.A., Peretti, B.M.P., Jaramillo, P.M.D., Teixeira, R.S.S., Dias, E.S., Félix, C.R., Filho, E.X.F.: Evaluation of hemicellulase production by plant-degrading fungi grown on agro-industrial residues. *Biodegradation* **21**, 815–824 (2010)
62. Ulloa, J.B., Weerd, J.H., Huisman, E.A., Verreth, J.A.J.: Tropical agricultural residues and their potential uses in fish feeds: the Costa Rican situation. *Waste Manag.* **24**, 87–97 (2004)
63. Xiros, C., Topakas, E., Katapodis, P., Christakopoulos, P.: Hydrolysis and fermentation of brewer's spent grain by *Neurospora crassa*. *Biores. Technol.* **99**, 5427–5435 (2008)
64. Schmitz, E., Karlsson, E.N., Adlercreutz, P.: Ultrasound assisted alkaline pre-treatment efficiently solubilises hemicellulose from oat hulls. *Waste Biomass Valorization* (2021). <https://doi.org/10.1007/s12649-021-01406-0>
65. Sun, R., Tomkinson, J.: Characterization of hemicelluloses obtained by classical and ultrasonically assisted extractions from wheat straw. *Carbohydr. Polym.* **50**, 263–271 (2002). [https://doi.org/10.1016/S0144-8617\(02\)00037-1](https://doi.org/10.1016/S0144-8617(02)00037-1)
66. Hersey, M.D.: Hints and suggestions to farmers. In: Hersey, M.D. *My work is that of conservation: An environmental biography of George Washington Carver*, pp. 124–159. Ugapress, Athens (2011)
67. Wolf, J., Gouvea, A., Silva, E.R.L., et al.: Métodos físicos e cal hidratada para manejo do cascudinho dos aviários. *Ciência Rural* **44**, 161–166 (2014)
68. Cunningham, A.B., Yang, X.: *Mushrooms in Forests and Woodlands: Resources Management, Values and Local Livelihoods*. Earthscan, Londres (2011)
69. Hikichi, S.E., Andrade, R.P., Dias, E.S., et al.: Biotechnological applications of coffee processing by-products. In: Galanakis, C.M. (ed.) *Handbook of Coffee Processing By-Products: Sustainable Applications*, pp. 221–244. Elsevier, Amsterdam (2017)
70. Kalač, P.: Proximate composition and nutrients. In: Kalač, P. (ed.) *Edible Mushrooms*, pp. 7–69. Academic Press, San Diego (2016)
71. Bonatti, M., Karnopp, P., Soares, H.M., Furlan, S.A.: Evaluation of *Pleurotus ostreatus* and *Pleurotus sajor-caju* nutritional characteristics when cultivated in different lignocellulosic wastes. *Food Chem.* **88**, 425–428 (2004)
72. Ogundele, G.F., Salawu, S.W., Abdulaheem, I.A., Bamidele, O.P.: Nutritional composition of oyster mushroom (*Pleurotus ostreatus*) grown on softwood (*Daniella oliveri*) sawdust and hardwood (*Anogeissus leiocarpus*) sawdust. *Br. J. Appl. Sci. Technol.* **20**, 1–7 (2017)
73. Zięba, P., Kała, K., Włodarczyk, A., et al.: Selenium and zinc biofortification of *Pleurotus eryngii* mycelium and fruiting bodies as a tool for controlling their biological activity. *Molecules* **25**, 1–18 (2020)
74. Solovyev, N., Prakash, N.T., Bhatia, P., et al.: Selenium-rich mushrooms cultivation on a wheat straw substrate from seleniferous area in Punjab, India. *J. Trace Elem. Med Biol.* **50**, 362–366 (2018)
75. Rathore, H., Sharma, A., Prasad, S., et al.: Yield, nutritional composition and antioxidant properties of *Calocybe indica* cultivated on wheat straw basal substrate supplemented with nitrogenous tree leaves. *Waste Biomass Valorization* **11**, 807–815 (2020)
76. Institute of Medicine: Food and Nutrition Board. *Dietary Reference Intakes*. National Academic Press, Washington, DC (2001)
77. Gąscka, M., Mleczek, M., Siwulski, M., et al.: The effect of selenium on phenolics and flavonoids in selected edible white rot fungi. *LWT Food Sci. Technol.* **63**, 726–731 (2015)
78. Carrasco-Gonzalez, J.A., Serna-Saldívar, S.O., Gutierrez-Urbe, J.A.: Mycochemical changes induced by selenium enrichment in *P. ostreatus* fruiting bodies. *J. Agric. Food Chem.* **65**, 4074–4082 (2017)

79. Gąsecka, M., Mleczek, M., Siwulski, M., et al.: Phenolic composition and antioxidant properties of *Pleurotus ostreatus* and *Pleurotus eryngii* enriched with selenium and zinc. *Eur. Food Res. Technol.* **242**, 723–732 (2016)
80. Gąsecka, M., Mleczek, M., Siwulski, M., et al.: Phenolic and flavonoid content in *Hericium erinaceus*, *Ganoderma lucidum*, and *Agrocybe aegerita* under selenium addition. *Acta Alimentaria* **45**, 300–308 (2016)
81. Frankel, E.N., Meyer, A.S.: The problem of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **80**, 1925–1941 (2000)
82. Rodriguez Estrada, A.E., Lee, H.J., Beelman, R.B., et al.: Enhancement of the antioxidants ergothioneine and selenium in *Pleurotus eryngii* var. *eryngii basidiomata* through cultural practices. *World J. Microbiol. Biotechnol.* **25**, 1597–1607 (2009)

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CAPÍTULO 3: Increased antioxidant activity of liver enzymes in piglets fed a diet enriched with selenized-yeast, and selenium-enriched or not *Pleurotus ostreatus* mushrooms

Increased antioxidant activity of liver enzymes in piglets fed a diet enriched with selenized-yeast, and selenium-enriched or not *Pleurotus ostreatus* mushrooms

Abstract

The enrichment of mushrooms with selenium can be used as a strategy to provide this mineral to the diet, since mushrooms are able to absorb selenium from the grown substrate. In this work, the selenium-enriched *Pleurotus ostreatus* mushroom and selenized-yeast were used in piglets diet at 0.3 ppm of selenium for 21 days to investigate the performance of animals, the bioavailability, and antioxidant activity of this mineral in plasma, measured as activity of glutathione peroxidase, catalase and superoxide dismutase, and malondialdehyde and nitric oxide contents. The selenium antioxidant activity was also analyzed in the liver. There was no difference in the performance of animals, and the feed conversion ratio ranged from 1.29 to 1.33. The use of selenium in piglet's diets also did not influence the enzymatic activity in plasma, and the activity of plasmatic glutathione peroxidase ranged from 75.74 to 79.09 U mL⁻¹. However, unlike the results found in the plasma, the use of selenium-enriched mushrooms or not and selenium-enriched yeast increased the enzymatic activity of all analyzed enzymes in the liver. The activity of hepatic glutathione peroxidase, for example, in animals that received the control diet (G1) was 14.55 U mL⁻¹, while it was 23.29, 26.51, and 23.13 U mL⁻¹, in the liver of animals that received mushrooms (G2), selenium-enriched mushrooms (G3) and selenized-yeast (G4), respectively. This increase in antioxidant activity in the liver is important, since it is one of the main organ metabolizers in the organism.

Keywords: selenized-mushroom, antioxidant activity, bioavailability, selenized-yeast, glutathione peroxidase.

Introduction

Selenium is an essential mineral for the maintenance of the homeostasis of organisms, mainly for the benefit of the immune system, muscle regeneration and reproduction (THOMSON, 2013). Several proteins involved in these processes are selenium-dependent enzymes, such as glutathione peroxidase (Gpx), whose active site is composed of selenocysteine and converts reactive oxygen species (ROS), naturally formed by metabolism, into other less reactive molecules (SHCHEDRINA et al., 2010; TAPIERO, TOWNSEND & TEW, 2003).

Beyond Gpx, superoxide dismutase (SOD), which catalyzes the formation of peroxides from the superoxide radical ($O_2^{\cdot -}$), and catalase (CAT), which converts these peroxides into water and oxygen, comprises the endogenous antioxidant system. However, the Gpx oxidizes glutathione (GSG) to exert this mechanism, and oxidized glutathione (GSH) is regenerated by the enzyme glutathione reductase (GR), which uses NADPH as a substrate (WATSON, PREEDY & ZIBADI, 2013). Since selenium is present in the active site of Gpx, its consumption increases this enzyme activity, and, consequently, influences the CAT and SOD response, once these enzymes act together (TÜRKER et al., 2011).

On the other hand, when selenium is limitant, the reduced activity of antioxidant enzymes can lead to an excess of ROS, reactive nitrogen species (RNS), such as malondialdehyde, which is a final product of lipid peroxidation, and nitric oxide (SOUZA et al., 2020). Low concentrations of nitric oxide protect the organism against ischemia and aid blood flow, while high amounts are related to toxicity, inflammation and, in more severe cases, septic shock (STOREY, 2004). The excess of these substances are associated with the occurrence of diabetes *mellitus*, cardiovascular disease and cancer. Keshan and Kashin-Back diseases, characterized by heart failure

and endemic osteoarthritis, respectively, are also related to low consumption of selenium (HATFIELD, 2001).

In some cases, the supplementation of the diet with the mineral is indicated, which can be performed with the use of inorganic (selenite and selenate) or organic (selenomethionine and selenocysteine) forms of selenium. The enrichment of foods, or biofortification is another alternative. Edible mushrooms could be used for this purpose, since fungi absorb and incorporate minerals into their fruiting bodies (DA SILVA et al., 2010; DA SILVA et al., 2012; SOUZA et al., 2021), converting inorganic forms of selenium into organic ones, which are commonly more bioavailable (ASSUNÇÃO et al., 2014). Other fungi to which selenium may be added are yeast, such as *Saccharomyces cerevisiae*, these organisms also convert inorganic into organic selenium forms and are widely used in animal feed (PRAUCHNER, 2014; ZHAO et al., 2017; CHEN et al., 2019).

Consumption of selenized mushrooms elevated selenium content in plasma (DA SILVA et al., 2010) and Gpx activity in the colon of mice (MASEKO et al., 2014). The activity of this enzyme in the liver and kidneys of diabetic mice was also elevated after consumption of these foods (LIU et al., 2015). The use of animal models with greater similarity to the human organism, like porcine (AJUWON, 2017), could help to elucidate the real benefits of consuming selenized mushrooms, helping to choose the best supplementation alternatives for this mineral in the human diet.

So, this manuscript describes the performance, antioxidant activity and selenium bioavailability in plasma, and antioxidant activity in liver of piglets feed with diets supplied with mushrooms (*Pleurotus ostreatus*) enriched or not with selenium, or selenized-yeast.

Material and methods

Mushrooms production

The mushrooms production was performed according to Souza et al. (2021), with some modifications. Briefly, the spawn was obtained by the grown of *P. ostreatus* (strain PLO 02) in sorghum grains previously cooked in water for 40 min and autoclaved at 121 °C for 1 h. Sugarcane bagasse was used as substrate for mushrooms cultivation. For this, this by-product was dried, crushed into pieces of ~2 cm, and immersed in a hydrated lime solution ($\text{Ca}(\text{OH})_2$) at 2 % (m/v) for 16 h, centrifuged (Britânia[®], 37501002) for 1 min at 600 rpm (50.4 g) to remove excess of lime solution. Then, 1 kg of this substrate was added into polypropylene bags and 10 mL of distilled water or sodium selenite solution at 25 mg kg⁻¹ of selenium were added to each bag. Finally, the spawn was inoculated and the bags were sealed and incubated at 25 °C until the substrates were completely covered by the mycelia. Then, thermal shock (10 °C) was performed for 24 h to induce fruiting. The bags were left at 23 °C and 80 % relative humidity until the mushroom harvesting, which was performed when the cap of the mushrooms was still facing down. The mushrooms were dehydrated in a food dehydrator at 55 °C (Meloni[®], Pratic Dryer) until constant weight and ground in a mini food processor (Palcookin[®], TK102) for addition to the feed.

Feed preparation

A mixture of corn and soybean brans was used as the basic diet for piglets (Group 1 - non supplemented control). It was formulated to have 3400 kcal kg⁻¹ of metabolizable energy (ME) and digestible lysine at 14.5 g kg⁻¹. The minimum contents of digestible

methionine + cysteine, threonine, tryptophan and valine, expressed as a percentage of digestible lysine, were 56, 63, 18 and 69 %, respectively, according to the ideal amino acid profile recommended by the Brazilian Tables for Poultry and Swine (ROSTAGNO et al., 2011). Corn bran was replaced by 1.53 g kg⁻¹ of mushroom powder (Group 2 - control supplemented with non-enriched mushrooms) and with selenium-enriched mushroom powder (Group 3). A mass of 0.3 g kg⁻¹ of selenized-yeast was added to the diets of piglets of Group 4. The selenized-yeast Sel-plex[®] (Alltech Inc., USA) was used as the positive control for the benefits of selenium supplementation in piglets diets, since it is regularly used for this purpose in animal production. It comprises an extract of cells of *Saccharomyces cerevisiae*, strain CNCM I-3060, grown on medium supplemented with sodium selenite (PRAUCHNER, 2014).

The amounts of selenized-yeast (Sel-plex[®]) and selenium-enriched mushroom powder were calculated based on their selenium content, which were 1000.00 and 196.00 µg g⁻¹, respectively, to meet the recommendation of the Food and Drug Administration of 0.3 ppm of selenium (FDA, 2017).

Experimental design

The experiment was approved by the Ethics Committee on the Use of Production Animals - CEUAP/UFV, process number 106/2018, and conducted at the experimental pig farm of the Department of Animal Science, at UFV.

Sixty four AGPIC 415 × Camborough piglets (Agroceres PIC, MG, Brazil), weaned at 21 days of age with an initial weight of 7.25 ± 0.64 kg were distributed in randomized blocks based on the initial weight, and feed during 21 days with diets according to the 4 treatments: G1 - control diet; G2 - diet containing non-enriched mushroom; G3 - diet

containing selenium-enriched mushrooms; G4 - diet containing selenized-yeast. Each treatment (group) was composed of 8 repetitions (pens), with 2 animals per pen, one male and one female.

The temperature inside the experimental room ranged from 25.5 to 28.0 °C. Water and feed were provided *ad libitum*.

Performance

The average of the weights of both animals of each pen (repetition) was considered for the calculations. The animals were weighed individually at 21 (beginning of the experiment), 35 and 42 days old (end of the experiment). The amounts of feed provided, leftovers and waste were also measured in the same period. The data were used for the calculation of average daily intake (ADI) (kg day^{-1}), average daily gain (kg day^{-1}) (ADG) and feed conversion ratio ($\text{FCR} = \text{ADI ADG}^{-1}$).

Antioxidant activity and selenium analyses

Sample collection and preparation

Blood was collected (5 mL) by puncturing the orbital sinus at 35 and 42 days old of one piglet per pen and the material placed in heparin tubes. The blood was kept under refrigeration and plasma was obtained by centrifugation at 3584 g for 10 min. At the end of the experiment, after a 12 h fasting, one piglet per pen was slaughtered. The piglet was rendered unconscious using head-only electrical stunning (240 V, 1.3 A) (ROCHA et al., 2012). Right after exsanguinated, the liver was taken and maintained at -

80 °C. Part of the organ collected was lyophilized, ground in a miniprocessor and sieved for selenium analysis. For the analysis of antioxidant activity of the liver, pieces of 100 mg were homogenized in 1 mL of phosphate buffer at 100 mM (pH 7.4). This homogenate was centrifuged at 3500 g and 4 °C, for 10 min, and 1 mL of the supernatant was removed and stored at -20 °C (SOUZA et al., 2018). The plasma was used directly for all analyses.

All analyses were performed using microplates, and absorbance readings were performed using a Multiskan GO[®] (Thermo Scientific) spectrophotometer.

Determination of Glutathione peroxidase (Gpx), catalase (CAT) and superoxide dismutase (SOD) activities

The Gpx, CAT and SOD activities were expressed in U mL⁻¹ (plasma) or U mg protein⁻¹ (liver). The total protein content was determined by the Bradford method (BRADFORD, 1976).

The Gpx activity was determined according to Cichoski et al. (2012) with some modifications. Briefly, 15 µL of plasma or 5 µL of liver homogenate + 10 µL of phosphate buffer (60 mM) with sodium azide (1.0 mM) and EDTA (0.5 mM), pH 7.0 (reaction buffer) were used. Sodium azide inhibits catalase activity (KEILIN & HARTREE, 1934). As a control, 15 µL of reaction buffer were used. The reaction medium was composed of 243 µL of reaction buffer with reduced glutathione (1.12 mM), NADPH (0.2 mM) and glutathione reductase (0.243 U).

To start the reaction, 42 µL of H₂O₂ at 0.72 mM were added and, after incubation for 10 min at 37 °C, the decay of NADPH absorbance was monitored at 340 nm for 5 min.

One unit of Gpx activity was defined as the amount of enzyme required to oxidize 1 nM of NADPH to NADP⁺ per minute, and was calculated using the equation 1:

(Equation 1)

$$U = \frac{\left[\frac{(\Delta Aa - \Delta Ac)}{t} * F \right]}{Q}$$

Where: ΔAa and ΔAc correspond to the decay of samples and control absorbances, respectively; t is the reaction time (min); Q is the protein content in the liver (mg); F is a constant used for converting absorbance per minute ($\Delta A/t$) into enzymatic units (U).

The variable F is calculated according to equation 2:

(Equation 2)

$$F = \left[\frac{\frac{Vr}{Va}}{0.00373} \right]$$

Where: Vr is the reaction volume, Va is the sample volume and 0.00373 is the molar extinction coefficient (in $\mu\text{M}^{-1} \text{cm}^{-1}$). The current NADPH molar extinction coefficient is $0.00622 \mu\text{M}^{-1} \text{cm}^{-1}$, but it was according to the optical path followed by the light on a microplate (0.6 cm) (MINGO, LÖTTERS & WAGNER, 2017).

The CAT activity was evaluated by measuring the kinetics of H_2O_2 decomposition, according to Aebi (1984). The SOD activity was estimated according to the pyrogallol method, based on the ability of this enzyme to catalyze the reaction of superoxide (O^{-2}) and hydrogen peroxide (H_2O_2) (DIETERICH et al., 2000).

Malondialdehyde (MDA) and nitric oxide (NO)

The MDA content was measured according to Buege & Aust (1978). For the analysis, 400 μL of thiobarbituric acid solution (trichloroacetic acid at 15 % m/v; thiobarbituric

acid at 0.375 % m/v and HCl at 0.25 N) were added to 200 μ L of plasma or liver homogenate. The reaction occurred at 90 °C, in a water bath, for 40 min. The microtubes were left at room temperature until equilibrium was obtained, and 600 μ L of butyl alcohol (P.A.) were added to the microtubes, which were centrifuged at 1200 g for 5 min, at room temperature, and 200 μ L of the supernatant were used for the absorbance reading. The formation of thiobarbituric acid reactive substances was monitored at 535 nm, and the malondialdehyde content (expressed in mg of protein) was calculated by the ratio of absorbance and the molar extinction coefficient of thiobarbituric acid ($\epsilon = 0.156 \mu\text{mol.L}^{-1}$).

The nitric oxide (NO) content was indirectly measured by the quantification of the nitrite/nitrate levels by Griess' standard reaction. In summary, 50 μ L of plasma or liver homogenate were incubated with the same volume of Griess' solution (sulfanilamide at 1 % m/v, naphthyl ethylenediamine dichlorhydrate at 0.1 % m/v and phosphoric acid at 2.5 % v/v) for 10 min. The absorbance was measured at 540 nm. The nitrite/nitrate levels were calculated with reference to a standard curve of sodium nitrite (NaNO_2), ranging from 0 to 100 μ M (TSIKAS, 2007).

Selenium quantification

Selenium contents were determined for mushrooms, selenized-yeast and in plasma. For the solids, 10 g of each supplement was ground in a cryogenic mill (Marconi, Brazil) with a self-contained liquid nitrogen bath. After 5 min of pre-cooling, 5 grinding cycles of 2 min each were employed, followed by a re-cooling stage of 1 min between cycles by switching off the magnetic field. Masses around 150 mg of ground substrates were digested in a microwave oven (Anton Paar, Multiwave 3000), using a diluted acid

mixture of 3.0 mL nitric acid (HNO_3) + 1.0 mL hydroxide peroxide (H_2O_2) (both purchased from Merck, Darmstadt, Germany) + 2.0 mL H_2O and the following heating program (step, temperature/ $^\circ\text{C}$, ramp/min, hold/min): 1, 140, 5, 1; 2, 180, 4, 5; and 3, 200, 4, 10. The selenium quantification was carried out with an atomic absorption spectrometer (AnalytikjenaAG, ZEE nit 60), equipped with a transversely heated graphite atomizer, pyrolytically coated graphite tube, and transversal Zeeman-effect background corrector. The spectrometer was operated with a hollow cathode lamp, using 10 μL of samples or analytical calibration solutions (5 to 90 $\mu\text{g L}^{-1}$ in 1.0 M HNO_3). Argon 99.998 % (v v $^{-1}$) (Air Liquide Brasil, Brazil) was used as protective and purge gas. The heating program used for the graphite furnace was the following (step, temperature/ $^\circ\text{C}$, ramp/s, hold/s): drying I, 100, 10, 15; drying II, 130, 10, 20; pyrolysis, 1200, 100, 20; atomization, 2300, 0, 5; and cleaning 2500, 1, 2. In all steps, Argon flow rate was 250 mL min^{-1} , except during atomization that was interrupted (OLIVEIRA & NAOZUKA, 2019). The results were expressed as micrograms of Se per g of samples. The total concentration of selenium in the plasma was measured according to the procedure proposed by Da Silva et al. (2010). The results were expressed as micrograms of selenium per liter of plasma.

Statistical analysis

The R software (version 3.6.2) was used for statistical analysis. Initially, the results were submitted to the normality test (Shapiro-Wilk) of variances and, when necessary, transformed according to Box & Cox (1964), and, after statistical analysis, converted to the original value. After analysis of variance (ANOVA – F test), Tukey test were used for compare the averages.

Results and discussion

Performance

The animals start feeding only on the second day of the experiment, due to stress caused by weaning, which has been demonstrated previously (MOESER, POHL & RAJPUT, 2017). There was no difference ($p>0.05$) in the ADG (kg day^{-1}), ADI (kg day^{-1}), and FCR (ADI ADG^{-1}), regardless of the feed composition provided for each group (Table 1), indicating that the use of mushrooms in the feed did not influence the performance of animal production. More important, this means that the mushrooms were palatable to the animals and did not harm them. The intake of selenomethionine (CAO et al., 2014) and a seleno-hydroxy-methionine analogue (CHAO et al., 2019), which are organic forms of this mineral, also did not affect the performance of piglets. A similar behavior was also observed for rats fed with spirulina, selenomethionine (CASES et al., 2001), and selenium-enriched *Agaricus bisporus* mushrooms (SPOLAR et al., 1999).

Table 1 – Performance of piglets fed with different sources of organic selenium

Parameter	Treatment				CV	p
	G1	G2	G3	G4		
BW – 21 d (kg)	7.23 ± 0.60	7.28 ± 0.73	7.26 ± 0.58	7.26 ± 0.66	2.84	0.97
BW – 35 d (kg)	10.01 ± 1.09	10.25 ± 1.09	9.77 ± 1.14	10.43 ± 1.00	7.92	0.39
BW – 42 d (kg)	13.15 ± 1.31	13.43 ± 1.38	13.18 ± 1.68	13.65 ± 1.22	8.28	0.78
21-35 d						
ADI (kg)	0.26 ± 0.07	0.28 ± 0.04	0.26 ± 0.03	0.28 ± 0.04	17.67	0.69
ADG (kg)	0.21 ± 0.06	0.21 ± 0.03	0.20 ± 0.03	0.23 ± 0.05	21.74	0.63
FCR	1.29 ± 0.08	1.32 ± 0.08	1.32 ± 0.08	1.29 ± 0.09	6.56	0.89
21-42 d						
ADI (kg)	0.37 ± 0.08	0.39 ± 0.05	0.37 ± 0.04	0.39 ± 0.05	14.52	0.83
ADG (kg)	0.28 ± 0.06	0.29 ± 0.04	0.30 ± 0.03	0.30 ± 0.05	14.96	0.76
FCR	1.32 ± 0.06	1.33 ± 0.02	1.29 ± 0.11	1.29 ± 0.06	5.27	0.70

G1 – control diet; G2 – diet with non-enriched mushrooms; G3 – diet with selenium-enriched mushrooms; G4 – diet with selenized-yeast; CV – coefficient of variation; p – p-value; BW – body weight; d – days; ADI – average daily intake; ADG – average daily gain; FCR – feed conversion ratio. For each parameter analyzed, the averages did not differ by F test ($p > 0.05$).

Selenium content in the plasma and antioxidant activity in plasma and liver

Feeding the animals with supplemented diets did not increase the antioxidant activity in the plasma (Table 2), but the liver presented an increase in this parameter (Table 3). This result was independent of the supplement being selenium-enriched or not. Since the piglets were not subjected to major stress conditions, and also did not have any disease induced or aggravated by the presence of ROS in the organism, the simple supplementation of the diet with a food rich in antioxidant compounds (phenolics and β -glucans) (LEE et al., 2017), as is the case of mushrooms, was already sufficient to generate an increase in the antioxidant capacity of the liver. However, in this case, selenium enrichment appears not to have been necessary. Possibly, the results would have been different if the animals had been subjected to some stressful condition or some induced disease, since in heat-stressed pregnant sows (CHEN et al., 2019) and in piglets contaminated with the herbicide Diquat (DOAN et al., 2020), after consuming selenium, their antioxidant activity in plasma presented an increase. When there is an increase in antioxidant activity in the stressed organism after the consumption of a certain food, it means that this product is helping the organism to combat the stress damage and this was also observed in the liver and kidneys of diabetic mice that consumed selenized mushrooms (LIU et al., 2015).

Table 2 – Selenium content, activity of enzymes glutathione peroxidase, catalase and superoxide dismutase, and of nitric oxide and malondialdehyde contents in the plasma of piglets

Parameter	Treatment				CV	p
	G1	G2	G3	G4		
35 d						
Se ($\mu\text{g L}^{-1}$)	17.88 \pm 4.50	14.51 \pm 9.15	20.69 \pm 9.55	19.24 \pm 5.86	42.49	0.43
Gpx (U mL ⁻¹)	67.96 \pm 6.77	70.91 \pm 4.36	63.27 \pm 9.23	66.62 \pm 8.97	11.50	0.28
Cat (U mL ⁻¹)	185.6 \pm 48.75	200.55 \pm 29.58	191.41 \pm 53.05	175.74 \pm 11.44	18.60	0.56
SOD (U mL ⁻¹)	4.17 \pm 2.02	3.05 \pm 2.58	4.31 \pm 1.39	3.78 \pm 3.10	62.73	0.73
NO (μM)	8.70 \pm 1.10	9.17 \pm 1.08	8.53 \pm 0.69	7.86 \pm 0.97	11.54	0.09
MDA (nM mg prot ⁻¹)	0.34 \pm 0.18	0.24 \pm 0.05	0.27 \pm 0.22	0.29 \pm 0.13	55.48	0.68
42 d						
Se ($\mu\text{g L}^{-1}$)	20.88 \pm 6.80	19.42 \pm 7.45	20.41 \pm 11.06	19.85 \pm 5.92	37.53	0.98
Gpx (U mL ⁻¹)	76.94 \pm 5.28	79.09 \pm 5.15	75.74 \pm 8.07	76.54 \pm 5.72	8.15	0.74
CAT (U mL ⁻¹)	148.39 \pm 32.57	170.39 \pm 58.23	162.00 \pm 36.16	159.81 \pm 37.82	26.39	0.78
SOD (U mL ⁻¹)	3.75 \pm 2.60	3.21 \pm 1.78	2.81 \pm 2.22	4.07 \pm 1.47	60.45	0.64
NO (μM)	8.67 \pm 2.42	8.11 \pm 1.44	8.13 \pm 1.63	7.13 \pm 0.59	19.52	0.28
MDA (nM mg prot ⁻¹)	0.30 \pm 0.27	0.23 \pm 0.15	0.18 \pm 0.08	0.19 \pm 0.06	73.27	0.44

G1 – control diet; G2 – diet with non-enriched mushrooms; G3 – diet with selenium-enriched mushrooms; G4 – diet with selenized-yeast; CV – coefficient of variation; p – p-value. prot = protein For each parameter analyzed, averages not differ significantly by F test ($p > 0.05$).

Table 3 – Activity of enzymes glutathione peroxidase, catalase and superoxide dismutase, and nitric oxide and malondialdehyde contents in the liver of piglets

Parameter	Treatment				CV	p
	G1	G2	G3	G4		
Gpx (U mg prot ⁻¹)	14.55 \pm 3.31 b	23.29 \pm 6.49 a	26.51 \pm 8.42 a	23.17 \pm 4.93 a	28.31	0.005
CAT (U mg prot ⁻¹)	16.18 \pm 3.90 b	23.27 \pm 4.55 a	29.28 \pm 12.35 a	26.22 \pm 4.96 a	30.41	0.008
SOD (U mg prot ⁻¹)	1.06 \pm 0.18 b	1.56 \pm 0.38 a	1.80 \pm 0.67 a	1.54 \pm 0.31 a	28.73	0.014
NO (μM)	11.98 \pm 3.97 a	7.67 \pm 1.13 b	7.56 \pm 1.02 b	7.45 \pm 1.72 b	25.51	0.002
MDA (nM mg prot ⁻¹)	0.43 \pm 0.09 a	0.31 \pm 0.09 a	0.43 \pm 0.18 a	0.39 \pm 0.14 a	33.65	0.246

G1 – control diet; G2 – diet with non-enriched mushrooms; G3 – diet with selenium-enriched mushrooms; G4 – diet with selenized-yeast; CV – coefficient of variation; p – p-value. prot = protein. For each parameter analyzed, averages with different letters differ significantly by Tukey test ($p < 0.1$).

Studies using other animal models had presented different results, even for non-stressed animals. For example, rats fed with diet containing selenium-enriched *P. ostreatus* at 0.15 ppm of selenium presented high content of this mineral in the plasma (680 $\mu\text{g L}^{-1}$), in comparison to the plasma of rats fed with control diet (269 $\mu\text{g L}^{-1}$) (DA SILVA et al., 2010). Similarly, when calves were fed with control diet or diet supplemented with selenium of mycelia from *L. edodes*, at 5 μg of Se per kg^{-1} of body weight, the selenium concentrations in the serum increased from 38.5 to 210.7 $\mu\text{g L}^{-1}$ (MUSZYŃSKA et al., 2020). The plasmatic Gpx activity was increased in horses fed with diets supplemented with 0.3 ppm of selenium from selenized-yeast (CALAMARI, FERRARI & BERTIN,

2009). On the other hand, Koyama et al. (2007) did not observe any increase in the Gpx activity in the plasma of mice when using selenomethionine at 0.4 ppm of selenium.

Also, in heat stressed pregnant sows, feed enriched with different concentrations of sodium selenite and selenized-yeast at 0.3 and 0.9 ppm of selenium, respectively, did not increase SOD activity and did not reduce MDA content in the plasma of these animals (CHEN et al., 2019). Rats with hypercholesterolemia were fed with diets supplemented with sodium selenite at 1.0 ppm of selenium and presented reduced nitric oxide content in the plasma (KANG, BANSAL & MEHTA, 1998).

Together, these studies indicate that diet supplementation with selenium did not alter the antioxidant activity in the plasma of most animals. It seems that the antioxidant activity alteration in the animals' plasma due to diet supplementation depends on several factors, being relevant the presence of diseases or environmental stresses and the amount and source of mineral supplied.

The maintenance of enzyme activities and selenium content in the plasma of the animals indicates that the diets were not harmful and this mineral from the mushroom and yeast has been metabolized by the liver and excreted, or transported to other sites, such as the kidney and loin, since organic selenium follows different routes and can even be randomly incorporated to replace methionine during the protein synthesis process (PRAUCHNER, 2014).

Nitric oxide (NO) is a vasodilator that facilitates blood flow, and its increased concentration is related to inflammatory processes (VIRÁG et al., 2003). NO is formed under the action of the enzyme nitric oxide synthase during the conversion of arginine into citrulline. When NO binds to superoxide (O_2^-), peroxynitrite ($ONOO^-$) is formed, which can cause damage to proteins and DNA (PRAUCHNER, 2014; VIRÁG et al., 2003). Malondialdehyde (MDA) is the final product of the decomposition of

polyunsaturated fatty acids, which, in excess, can contribute to the emergence of cardiovascular and hepatic diseases, in addition to diabetes, Alzheimer's, Parkinson's and cancer (AYALA, MUÑOZ & ARGÜELLES, 2014). Therefore, NO and MDA can be used as markers to determine if foods, such as selenium-enriched mushrooms, have any toxicity to animal organisms. The feeding of piglets with unenriched mushrooms or selenized compounds, regardless of origin, reduced NO levels in the liver but not in plasma.

In the liver, the Gpx activity was increased regardless of the enrichment of the mushrooms with selenium (Table 3). This increase in enzyme activity in G2 can be due to the presence of phytochemical compounds such as phenolic, beta-glucans and others, which induce the production of antioxidant enzymes and glutathione (LEE et al., 2017). For example, in studies with elderly rats that received an intraperitoneal application of *P. ostreatus* extract there was an increase in the expression of the enzyme catalase, and in the activity of enzymes related to the redox system of glutathione (JAYAKUMAR et al., 2010a; JAYAKUMAR et al., 2010b; JAYAKUMAR, THOMAS & GERALDINE, 2007). In addition, the consumption of this mushroom has reduced hypercholesterolemia and hyperglycemia in rats, mice and rabbits (BOBEK & GALBAY, 1999; BOBEK, OZDÍN & GALBAY, 1998; RAVI et al., 2013). In mice that suffered hepatic injury from acetaminophen injection, the supplementation with 10 % *P. ostreatus* was able to maintain constant the MDA content and enzyme activity of Gpx and SOD in the liver (NAGUIB et al., 2014).

The increase in antioxidant activity in the liver from G3 and G4 groups can be due to the selenium, and phytochemical compounds of mushrooms and yeast (LEE et al., 2017). The supplementation of diet with selenized-yeast at 0.2 ppm of Se, for example, increased the hepatic Gpx activity in broiler chicks (ZHAO et al., 2017) and in chickens

contaminated with cadmium this supplement at 0.5 ppm of Se reduced nitric oxide content in liver (WANG et al., 2020). Other selenium-enriched sources increased the hepatic antioxidant activity. For example, selenium-enriched ramps (*Allium tricoccum*) at 0.1 and 0.15 ppm of selenium increased the antioxidant activity (Gpx) in the liver of rats (WHANGER et al., 2000) and in the liver (Gpx and CAT activities) of broilers supplemented with selenium-enriched bacterial biomass (*Klebsiella pneumoniae*) at 0.3 ppm of selenium (DALIA et al., 2017).

According to the data obtained in this study and those obtained by the various authors mentioned above, biomarkers of antioxidant activity in animals, such as Gpx, CAT, SOD, NO and MDA are usually affected by the ingestion of selenium, especially its activities and concentrations in the liver. The form (organic and inorganic), method of administration, content of the mineral and needs of the organism greatly affect how this mineral will be metabolized and distributed. In the case of selenium depletion, for example, the brain, reproductive organs and endocrine glands will be prioritized in the reception of the metabolized mineral, while the activity of the Gpx of other parts of the organism will decrease. On the contrary, mineral intake is constantly increased, the excretion rate increases, especially by urine (PRAUCHNER, 2014).

Conclusion

The diets with selenium-enriched mushrooms or not and selenized-yeast did not harm piglet performance or plasma antioxidant activity. The intake of all diets and plasma selenium content were similar, indicating that the diets were palatable, and that excess selenium provided by the diet may have been excreted or transported to piglet organs and tissues. This transport may have occurred because there was an increase in the antioxidant activity of the animals' liver in the groups fed with selenium-enriched mushrooms and selenized-yeast. There was also an increase in liver antioxidant activity of the animals fed with non-selenized mushrooms, and in this case, the increase may have occurred due to the presence of compounds such as phenolics and β -glucans in the mushrooms.

REFERENCES

- AEBI, H. Catalase *in vitro*. **Methods in enzymology**, Florida, v. 105, p. 121-126, 1984.
- AJUWON, K. M. The pig model for the study of obesity and associated metabolic diseases. In: SCANIS, C. G.; HILL, R. A. (Eds). **Biology of domestic animals**. 370 p. Boca Raton: CRC Press, 2017. Cap. 2, p. 18-47.
- ASSUNÇÃO, L. S.; DA SILVA, M. C. S.; FERNANDEZ, M.G.; GARCÍA-BARRETA, T.; GOMÉZ-ARIZA, J. L.; BAUTISTA, J.; KASUYA, M. C. M. Speciation of selenium in *Pleurotus ostreatus* and *Lentinula edodes* mushrooms. **Journal of biotechnology letters**, v. 5, n. 1, p. 79-86, 2014.
- AYALA, A.; MUÑOZ, M. F.; ARGÜELLES, S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. **Oxidative medicine and cellular longevity**, v. 2014, p. 1-32, 2014.
- BOBEK, P.; GALBAVY, S. Hypocholesterolemic and antiatherogenic effect of oyster mushroom (*Pleurotus ostreatus*) in rabbits. **Nahrung**, v. 43, 339–342, 1999.
- BOBEK, P.; OZDIN, L.; GALBAVY, S. Dose- and time dependent hypocholesterolemic effect of oyster mushroom (*Pleurotus ostreatus*) in rats. **Nutrition**, v. 14, 282–286, 1998.
- BOX, G. E. P.; COX, D. R. An analysis of transformations. **Journal of the royal statistical society**. Series B (Methodological), v. 26, n. 2, p. 211-252, 1964.
- BRADFORD, M. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein- dye-binding. **Analytical biochemistry**, v. 72, p. 248-254, 1976.
- BUEGE, J. A.; AUST, S. D. Microsomal lipid peroxidation. **Methods in enzymology**, v. 52, p. 302-310, 1978.

- CALAMARI, L.; FERRARI, A.; BERTIN, G. Effect of selenium source and dose on selenium status of mature horses. **Journal of Animal Science**, v. 87, p. 167–178, 2009.
- CAO, J.; GUO, F.; ZHANG, L.; DONG, B.; GONG, L. Effects of dietary selenomethionine supplementation on growth performance, antioxidant status, plasma selenium concentration, and immune function in weaning pigs. **Journal of Animal Science and Biotechnology**, v. 5, n. 1, p. 1-7, 2014.
- CASES, J.; VACCHINA, V.; NAPOLITANO, A.; CAPORICCIO, B.; BESANÇON, P.; LOBINSKI, R.; ROUANET, J. M. Selenium from Selenium-Rich Spirulina Is Less Bioavailable than Selenium from sodium selenite and selenomethionine in selenium-deficient rats. **The Journal of Nutrition**, v. 131, n. 9, sept. 2001, p. 2343–2350, 2001.
- CHAO, Y.; YU, B.; HE, J.; HUANG, Z.; MAO, X.; LUO, J.; LUO, Y.; ZHENG, P.; YU, J.; CHEN, D. Effects of different levels of dietary hydroxy-analogue of selenomethionine on growth performance, selenium deposition and antioxidant status of weaned piglets. **Archives of Animal Nutrition**, v. 73, n. 5, p. 374-383, 2019.
- CHEN, J.; ZHANG, F.; GUAN, W.; SONG, H.; TIAN, M.; CHENG, L.; SHI, K.; SONG, J.; CHEN, F.; ZHANG, S. Increasing selenium supply for heat-stressed or actively cooled sows improves piglet preweaning survival, colostrum and milk composition, as well as maternal selenium, antioxidant status and immunoglobulin transfer. **Journal of Trace Elements in Medicine and Biology**, v. 52, p. 89–99, 2019.
- CICHOSKI, A. J.; ROTTA, R. B.; SCHEUERMANN, G.; CUNHA JÚNIOR, A.; BARIN, J. S. Investigation of glutathione peroxidase activity in chicken meat

under different experimental conditions. **Food Science and Technology**, Campinas, v. 32, n. 4, p. 661-667, out.-dez. 2012.

DALIA, A.; LOH, T.; SAZILI, A.; JAHROMI, M.; SAMSUDIN, A. The effect of dietary bacterial organic selenium on growth performance, antioxidant capacity, and selenoproteins gene expression in broiler chickens. **BMC Veterinary Research**, v.13, n. 254, 2017.

DA SILVA, M. C. S.; NAOZUKA, J.; LUZ, J. M. R.; ASSUNÇÃO, L. S.; OLIVEIRA, P. V.; VANETTI, M. C. D.; BAZZOLLI, D. M. S.; KASUYA, M. C. M. Enrichment of *Pleurotus ostreatus* mushrooms with selenium in coffee husks. **Food Chemistry**, v. 131, n. 2, p. 558-563, mar. 2012.

DA SILVA, M. C. S.; NAOZUKA, J.; OLIVEIRA, P. V.; VANETTI, M. C. D.; BAZZOLLI, D. M. S.; COSTA, N. M. B.; KASUYA, M. C. M. *In vivo* bioavailability of selenium in enriched *Pleurotus ostreatus* mushrooms. **Metallomics**, v. 2, p. 162-166, jan. 2010.

DIETERICH, S.; BIELIGK, U.; BEULICH, K.; HASENFUSS, G.; PRESTLE, J. Gene expression of antioxidative enzymes in the human heart: increased expression of catalase in the end-stage failing heart. **Circulation**, v. 101, p. 33-39, 2000.

DOAN, N.; LIU, Y.; XIONG, X. et al. Organic selenium supplement partially alleviated diquat-induced oxidative insults and hepatic metabolic stress in nursery pigs. **British Journal of Nutrition**, v. 124, n.1, p. 23-33, 2020.

FOOD AND DRUG ADMINISTRATION (FDA) - **Code of Federal Regulations Title**

21. 2017. Disponível em:

[https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.](https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=573.920)

920. Acesso em: 13 jun. 2018.

HATFIELD, D. L. **Selenium: Its molecular biology and role in human health**. 1 ed.

Boston: Kluwer Academic Publishers, 2001. 352 p.

JAYAKUMAR, T.; GERALDINE, P.; THOMAS, P. A. Protective effect of an extract of the oyster mushroom, *Pleurotus ostreatus*, on antioxidants of major organs of aged rats. **Experimental gerontology**, v. 42, p. 183-191, 2007.

JAYAKUMAR, T.; THOMAS, P. A.; SHEU, J. R.; GERALDINE, P. An extract of the oyster mushroom, *Pleurotus ostreatus*, increases catalase gene expression and reduces protein oxidation during ageing in rats. **Chinese journal of integrative medicine**, v. 8, n.8, p. 774–780, 2010a.

JAYAKUMAR, T.; THOMAS, P. A.; RAMESH, E.; GERALDINE, P. An extract of the mushroom, *Pleurotus ostreatus*, bolsters the glutathione redox system in various organs of aged rats. **Journal of medicinal food**, v. 13, n. 4, p. 1–8, 2010b.

KANG, B. P. S.; BANSAL, M. P.; MEHTA, U. Selenium supplementation and diet induced hypercholesterolemia in the rat: changes in lipid levels, malonyldialdehyde production and the nitric oxide synthase activity. **General physiology and biophysics**, v. 17, p. 71-78, 1998.

KOYAMA, H.; TERADA, A.; YOSHIDA, M.; NAKADA, K.; ABDULAH, R.; SATOH, H. Cysteine supplementation reduces the bioavailability of selenomethionine in mice. **European journal of clinical nutrition**, v. 2, p. 38–43, 2007.

KEILIN, D.; HARTREE, E. F. Inhibitors of catalase reaction. **Nature**, v. 134, p. 933, 1934.

LEE, M. T.; LIN, W. C.; YU, B.; LEE, T. T. Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals — A review. **Asian-australasian journal of animal sciences**, v. 30, n. 3, p. 299-308, 2017.

- LIU, Y.; LI, C.; LUO, X. et al. Characterization of selenium-enriched mycelia of *Catathelasma ventricosum* and their antihyperglycemic and antioxidant properties. **Journal of Agricultural and Food Chemistry**, v. 63, p. 562–568, 2015.
- MASEKO, T.; HOWELL, K.; DUNSHEA, F. R.; NG, K. Selenium-enriched *Agaricus bisporus* increases expression and activity of glutathione peroxidase-1 and expression of glutathione peroxidase-2 in rat colon. **Food chemistry**, 146, 327–333, 2014.
- MINGO, V.; LÖTTTERS, S.; WAGNER, N. The impact of land use intensity and associated pesticide applications on fitness and enzymatic activity in reptiles - A field study. **Science of the Total Environment**, v. 590, p. 114-124, 2017.
- MOESER, A. J.; POHL, C. S.; RAJPUT, M. Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. **Animal Nutrition**, v. 3, n. 4, p. 313-321, 2017.
- MUSZYŃSKA B.; SZACAWA E.; BEDERSKA-ŁOJEWSKA, D. et al. Preliminary study on Se-enriched *Lentinula edodes* mycelium as a proposal of new feed additive in selenium deficiency. **Plos One**, v. 15, p. 1-19, 2020.
- NAGUIB, Y. M.; AZMY, R. M.; SAMAKA, R. M.; SALEM, M. F. *Pleurotus ostreatus* opposes mitochondrial dysfunction and oxidative stress in acetaminophen-induced hepato-renal injury. **BMC complementary and alternative medicine**, v. 14, n.1, p. 1-12, 2014.
- OLIVEIRA, A. P.; NAOZUKA, J. Preliminary results on the feasibility of producing selenium-enriched pink (*Pleurotus djamor*) and white (*Pleurotus ostreatus*) oyster mushrooms: Bioaccumulation, bioaccessibility, and Se-proteins distribution. **Microchemical Journal**, v. 145, p. 1143–1150, 2019.

- PRAUCHNER, C. A. **A importância do selênio para a agropecuária e saúde humana**. Editora UFSM: Santa Maria, 2014. 376 p.
- RAVI, B.; RENITTA, R. E.; PRABA, M.L.; ISSAC, R. NAIDU, S. Evaluation of antidiabetic potential of oyster mushroom (*Pleurotus ostreatus*) in alloxan-induced diabetic mice. **Immunopathology in toxicology and drug development**, v. 35, n. 1, p. 101-109, 2013.
- ROCHA, G. C.; DONZELE, J. L.; OLIVEIRA, R. F. M.; SILVA, F. C. O.; KIEFER, C.; BRUSTOLINI, P. C.; PEREIRA, C. M. C.; ALEBRANTE, L. Avaliação dos níveis de zeólita em dietas para suínos em fase de crescimento e terminação. **Revista brasileira de zootecnia**, v. 41, n. 1, p. 111-117, 2012.
- ROSTAGNO, H. S. **Brazilian Tables for Poultry and Swine: Composition of Feedstuffs and Nutritional Requirements**. 3 ed., Viçosa: Editora UFV, 2011. 251 p.
- SHCHEDRINA, V.; ZHANG, Y.; LABUNSKYY, V.; HATFIELD, D.; GLADYSHEV V. Structure–function relations, physiological roles, and evolution of mammalian ER-resident selenoproteins. **Journal antioxidants and redox signaling**, v. 12, n. 7, p. 839-849, apr. 2010.
- SOUZA, A. C. F.; BASTOS, D. S. S.; SANTOS, F. C.; SERTORIO, M. C.; ERVILHA, L. O. G.; GONÇALVES, R. V.; OLIVEIRA, L. L.; MACHADO-NEVES, M. Arsenic aggravates oxidative stress causing hepatic alterations and inflammation in diabetic rats. **Life sciences**, v. 209, p. 472-480, 2018.
- SOUZA, A. C. F.; ERVILHA, L. O. G.; COIMBRA, J. L. P.; BASTOS, D. S. S.; GUIMARÃES, S. E. F.; MACHADO-NEVES, M. Reproductive disorders in female rats after prenatal exposure to sodium arsenite. **Journal of applied toxicology**, v. 40, p. 214-223, 2020.

- SOUZA, D. F.; DA SILVA, M. C. S.; ALVES, M. P. et al. By-products as substrates for production of selenium-enriched *Pleurotus ostreatus* mushrooms. **Waste and Biomass Valorization**, 2021. <https://doi.org/10.1007/s12649-021-01586-9>.
- SPOLAR, M. R.; SCHAFFER, E. M.; BEELMAN, R.B.; MILNER, J. A. Selenium-enriched *Agaricus bisporus* mushrooms suppress 7,12-dimethylbenz[a]anthracene bioactivation in mammary tissue. *Cancer Letters*, v. 138, p. 145–150, 1999.
- STOREY, K. B. **Functional Metabolism: Regulation and Adaptation**. Hoboken: John Wiley & Sons, Inc, 2004. 616 p.
- TAPIERO, H.; TOWNSEND, D. M.; TEW, K. D. The antioxidant role of selenium and seleno-compounds. **Biomedicine & Pharmacotherapy**, v. 57, n. 3, p. 134-144. 2003.
- THOMSON, C. D. Selenium. In: CABALLERO, B.; ALLEN, L.; PRENTICE, A. **Encyclopedia of Food Sciences and Nutrition**. 3 ed. (v. 4). Baltimore: Academic Press, 2013. Cap. 28. p. 188.
- TSIKAS, D. Analysis of nitrite and nitrate in biological fluids by assays based on the Griess reaction: Appraisal of the Griess reaction in the l-arginine/nitric oxide area of research. **Journal of chromatography B**, v. 851, p. 51-70. 2007.
- TÜRKER Y.; NAZIROĞLU M.; GÜMRAL N. et al. Selenium and L-carnitine reduce oxidative stress in the heart of rat induced by 2.45-GHz radiation from wireless devices. **Biological Trace Element Research**, v. 143, p. 1640-1650, 2011.
- VIRÁG, L.; SZABÓ, É.; GERGELY, P.; SZABÓ, C. Peroxynitrite-induced cytotoxicity: mechanism and opportunities for intervention. **Toxicology letters**, v. 140-141, p.113-124, 2003.
- WANG, Y.; LIU, J.; CHEN, R.; QI, M.; TAO, D.; XU, S. The Antagonistic effects of selenium yeast (SeY) on cadmium-induced inflammatory factors and the heat

shock protein expression levels in chicken livers. **Biological trace element research**, v. 198, p. 260-268, 2020.

WATSON, R. R.; PREEDY, V. R.; ZIBADI, S. **Polyphenols in Human Health and Disease**. London: Academic Press, 2013. 1488 p.

WHANGER, P. D.; IP, C.; POLAN, C. E.; UDEN, P. C.; WELBAUM, G.

Tumorigenesis, metabolism, speciation, bioavailability and tissue deposition of selenium in selenium-enriched ramps (*Allium tricoccum*). **Journal of agricultural and food chemistry**, v. 48, p. 5723-5730, 2000.

ZHAO, L.; SUN, L.-H.; HUANG, J.-Q.; BRIENS, M.; QI, D.-S.; XU, S.-W.; LEI, X.G.

A novel organic selenium compound exerts unique regulation of selenium speciation, selenogenome, and selenoproteins in broiler chicks. **The journal of nutrition**, v. 147, p. 789–797, 2017.

CONSIDERAÇÕES FINAIS

Os efeitos do uso do selênio no meio de cultivo de cogumelos diferem a depender da dose de selênio adicionada, do substrato e da espécie do cogumelo. A adição de selênio pode aumentar o rendimento de produção, tendo elevado em 20 % a eficiência biológica de *P. ostreatus* cultivado em cascas de café enriquecidas com 12,7 mg kg⁻¹ de selênio. O mineral é capaz também de aumentar a atividade antioxidante dos cogumelos devido a um aumento na síntese por eles de determinados compostos, como os fenólicos e β-glucanos, por exemplo. Nesse caso, já foram reportados aumentos de até 70 % na atividade antioxidante de cogumelos de *G. lucidum*. Um dos principais nutrientes presentes nos cogumelos, as proteínas, pode também ter sua síntese aumentada devido ao cultivo desses fungos na presença de selênio, como ocorreu no cultivo de cogumelos de *C. indica*, cujo aumento no conteúdo de proteínas foi de 71 % quando cultivado em palha de trigo a 10 mg kg⁻¹ de selênio. O selênio presente nos cogumelos enriquecidos está disponível principalmente em formas orgânicas e, por isso, é facilmente absorvido quando ingerido. No corpo, ele pode se tornar bioativo, elevando a atividade da enzima glutationa-peroxidase em alguns órgãos e reduzindo a concentração de radicais livres.

Neste trabalho, mostramos que o uso de tratamento alcalino do substrato inviabiliza o uso de bagaço de malte em maiores proporções, pela manutenção do pH deste substrato mesmo após o tratamento com solução (Ca(OH)₂) a 2 %. Por outro lado, foi possível usar esse método para tratamento de substrato composto por 25 % de bagaço de malte em combinação com bagaço de cana-de-açúcar (75 %), ou composto exclusivamente por este último, para produção de cogumelos de *P. ostreatus* enriquecidos com selênio a 25 mg kg⁻¹. Os cogumelos produzidos apresentaram elevados teores de proteínas (17,07 a 23,50 %) e de fibras (11,13 a 12,08 %). O uso de bagaço de cana-de-açúcar puro proporcionou cogumelos com maior concentração de selênio, rendimento e atividade

antioxidante nos corpos de frutificação. Esse subproduto agroindustrial é produzido em grande quantidade no Brasil e em outros países e a sua imersão em solução de cal hidratada é um processo de desinfestação mais barato que a esterilização, por isso, pode ser usado para produzir *P. ostreatus* com baixo custo.

Após a produção em bagaço de cana, os cogumelos de *P. ostreatus* enriquecidos ou não com selênio foram usados para enriquecimento da dieta de suínos. Leveduras selenizadas foram também utilizadas para o mesmo fim. As dietas contendo os cogumelos foram palatáveis aos animais e não prejudicaram o ganho de peso (0,28 a 0,30 kg por dia), mesmo em comparação aos animais que ingeriram dietas contendo leveduras selenizadas. Nenhuma dieta alterou o equilíbrio plasmático, indicando que o excesso de selênio das dietas suplementadas tenha sido conduzido para os órgãos e tecidos ou excretado. Isso foi confirmado pela maior atividade antioxidante no fígado dos suínos alimentados com dieta suplementada em comparação à atividade no fígado dos animais alimentados com dieta não suplementada. Nesse caso, a suplementação levou ao aumento da atividade das enzimas glutathione peroxidase, catalase e superóxido dismutase e foi independente de a suplementação ter sido realizada com cogumelos selenizados ou não, ou com leveduras selenizadas. A maior atividade antioxidante no fígado indica que o órgão apresentou maior capacidade de combate a radicais livres, o que pode auxiliar na redução do aparecimento de doenças como o câncer.

Assim, mostramos que o consumo de cogumelos comestíveis contribui para o aumento da atividade antioxidante, o que combate algumas doenças, como o câncer, o diabetes *mellitus* e doenças cardiovasculares. Assim, esses alimentos podem ser produzidos e biofortificados a partir de substratos e métodos de baixo custo, e isso poderia ser, portanto, uma alternativa para a implementação de políticas sociais de suplementação da dieta humana e animal em países onde a deficiência de selênio é uma realidade.