LEANDRO DE SOUZA LOPES

COGUMELOS ENRIQUECIDOS COM LÍTIO: BIOACESSIBILIDADE, BIODISPONIBILIDADE E EFEITO NA MICROBIOTA INTESTINAL DE SUÍNOS

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

Orientadora: Maria Catarina Megumi Kasuya

Coorientadores: Marliane de Cássia S. da Silva
Gabriel Cipriano Rocha

VIÇOSA - MINAS GERAIS
2022

Ficha catalográfica elaborada pela Biblioteca Central da Universidade Federal de Viçosa - Campus Viçosa

T
L864c
2022

Lopes, Leandro de Souza, 1985-
Cogumelos enriquecidos com lítio: bioacessibilidade, biodisponibilidade e efeito na microbiota intestinal de suínos / Leandro de Souza Lopes. – Viçosa, MG, 2022.
1 tese eletrônica (95 f.): il.

Texto em português e inglês.
Orientador: Maria Catarina Megumi Kasuya.
Tese (doutorado) - Universidade Federal de Viçosa, Departamento de Microbiologia, 2022.
Inclui bibliografia.
DOI: https://doi.org/10.47328/ufvbbt.2022.518
Modo de acesso: World Wide Web.

1. Cogumelos - Uso terapêutico. 2. Lítio - Uso terapêutico. 3. Flora microbiana. 4. Stress oxidativo. 5. Fungos. 6. Suínos. 7. *Pleurotus sp.*. I. Kasuya, Maria Catarina Megumi, 1961-. II. Universidade Federal de Viçosa. Departamento de Microbiologia. Programa de Pós-Graduação em Microbiologia Agrícola. III. Título.

CDD 22. ed. 579.6163

Bibliotecário(a) responsável: Euzébio Luiz Pinto CRB-6/3317

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APROVADA: 29 de julho de 2022

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AGRADECIMENTOS

À Universidade Federal de Viçosa e ao Programa de Pós-graduação em Microbiologia Agrícola pela oportunidade de realização do curso de Doutorado.

Às agências financiadoras CNPq, Capes e Fapemig pelo apoio financeiro para o desenvolvimento desta pesquisa e pela concessão da bolsa de estudos. O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Código de Financiamento 001.

À professora Maria Catarina, por ter aceitado me orientar, pelos ensinamentos acadêmicos e crescimento humano.

À Marliane, pela paciência, ensinamentos e disponibilidade sempre que precisei.

Ao grupo de pesquisa Cogumelo que me acolheu como uma família.

À Juliana e o BIOTAN pela paciência e contribuição neste trabalho.

À família Micorriza por todo aprendizado, pelo agradável convívio e por todos os momentos de descontração tornando essa jornada muito mais leve. Obrigado pelos conselhos e cafés, Camila, Emiliane, Betânia, Rita, Thiago, Nayron, Tomás! E obrigado aos irmãos, Fernando e Bárbara, que a vida me deu.

Ao Professor Gabriel e toda sua equipe da suinocultura do Departamento de Zootecnia pela ajuda no desenvolvimento deste projeto.

À psiquiatra dra. Kelly, pelas sugestões e apoio a este projeto.

À minha família por todo apoio durante essa longa jornada acadêmica.

A Deus por ter me dado força e permitido alcançar essa conquista tão sonhada.

Muito Obrigado!

RESUMO

LOPES, Leandro de Souza, D.Sc., Universidade Federal de Viçosa, julho de 2022. **Cogumelos enriquecidos com lítio: bioacessibilidade, biodisponibilidade e efeito na microbiota intestinal de suínos.** Orientadora: Maria Catarina Megumi Kasuya. Coorientadores: Marliane de Cássia Soares da Silva e Gabriel Cipriano Rocha.

Os cogumelos apresentam alto potencial para serem utilizados como fonte de lítio (Li), mas a bioacumulação de Li e outros minerais, status redox e biodisponibilidade são específicos para cada espécie, dependendo da dosagem e do tipo de sal utilizado. Cogumelos enriquecidos com Li podem tornar esse elemento mais bioacessível ao organismo quando o comparamos com o sal Li_2CO_3, mas ainda não há trabalhos publicados mostrando a biodisponibilidade *in vivo* desse mineral. Em indivíduos que fazem uso de medicamentos psiquiátricos a base de Li no tratamento de doenças como transtorno bipolar, depressão e ansiedade, a microbiota intestinal sofre mudanças em sua composição, cuja mudança pode estar relacionada aos efeitos desses medicamentos, porém há poucas informações a respeitos dos efeitos do Li na composição microbiona intestinal. Assim, o objetivo deste trabalho foi avaliar o bioacúmulo de Li em *Pleurotus ostreatus* (PLO 02) e *Pleurotus djamor* (PLO 13), além de avaliar a atividade oxidante, co-acúmulo de minerais e bioacessibilidade; avaliar também a biodisponibilidade *in vivo* de Li em cogumelos enriquecidos de *P. djamor* em suínos, além de parâmetros como status redox, estresse e acúmulo desse mineral nos tecidos dos animais e efeitos do Li na comunidade microbiona intestinal de suínos. Nossos resultados mostraram que PLO 02 é mais sensível a altas concentrações de Li do que PLO 13, no qual PLO 13 apresentou maior taxa de produção de cogumelos. Observou-se correlação positiva entre o acúmulo de Li e outros minerais presentes no substrato e o efeito do Li no estado redox do fungo. A bioacessibilidade em ambos os fungos foi maior nos cogumelos enriquecidos com Li quando comparado ao comprimido de carbonato de lítio medicinal, e esses cogumelos podem co-acumular minerais e aumentar a atividade oxidativa. No experimento *in vivo* com suínos, *P. djamor* enriquecido com Li foi uma fonte mais biodisponível de Li quando comparados à forma Li_2CO_3, e os cogumelos enriquecidos com Li melhoraram os efeitos das enzimas oxidativas, e em alguns tecidos, os cogumelos enriquecidos com Li apresentaram menor dano oxidativo quando comparados às suas correspondentes dosagens na forma de Li_2CO_3. Pela análise dos dados do sequenciamento das amostras intestinais verificamos que o Li influencia os índices de diversidade alfa, mostrando diferença nos índices de Simpson, Shannon e Chao-1 no cólon e Chao-1 nas fezes,

dependendo do tratamento, particularmente aqueles tratamentos que receberam lítio, aumentando a diversidade, quando comparados aos tratamentos que não receberam nenhuma forma de lítio. O agrupamento taxonômico das variantes da sequência amplicon (ASVs) mostrou que os táxons com maior abundância relativa podem variar entre o íleo, cólon e fezes, com predominância de filos como *Firmicutes*, *Bacteroidota* e *Proteobacteria* nos tratamentos que recebem lítio. Muitos grupos de microrganismos importantes para a saúde animal como *Lactobacillus*, *Ruminococcaceae*, *Enterorhabdu*s, *Muribaculaceae* e *Coprococcus* tem sua abundância relativa aumentada nos animais que receberam Li e ou Cogumelo enriquecido com Li. Os fungos bioacumuladores de minerais, como o *P. djamor* e *P. ostreatus*, apresentam potencial para serem utilizados na saúde humana e animal, pois além de aumentar a disponibilidade de Li, diminui os efeitos tóxicos em indivíduos que fazem o seu uso terapêutico e aumentando populações de microorganismos importantes que mantêm a homeostase do intestino.

Palavras-chave: *Pleurotus* spp. Estresse oxidativo. Carbonato de lítio. Metataxonomia. Ácidos orgânicos.

ABSTRACT

LOPES, Leandro de Souza, D.Sc., Universidade Federal de Viçosa, July, 2022. **Lithium-enriched mushrooms: bioaccessibility, bioavailability and effect on the intestinal microbiota of swine.** Advisor: Maria Catarina Megumi Kasuya. Co-advisors: Marliane de Cássia Soares da Silva and Gabriel Cipriano Rocha.

Mushrooms have a high potential to be used as a source of lithium (Li), but the bioaccumulation of Li and other minerals, redox status and bioavailability are specific for each species, depending on the dosage and type of salt used. Li-enriched mushrooms can make this element more bioaccessible to the body when compared to its Li_2CO_3 salt, but there are still no published works showing the in vivo bioavailability of this mineral. It is known that intestinal microbiota undergoes changes in its composition in individuals who use psychiatric drugs in the treatment of diseases, and that this change in the microbiota may be related to the therapeutic effects of these drugs, but there is little information regarding the effects of lithium on the intestinal microbial composition. So, the objective of our work was to evaluate the bioaccumulation of Li in *Pleurotus ostreatus* (PLO 02) and *Pleurotus djamor* (PLO 13), in addition to evaluating the oxidative activity, co-accumulation of minerals and bioaccessibility; besides, to evaluate the *in vivo* bioavailability of Li-enriched mushrooms of *P. djamor* in swine, in addition to parameters such as redox status, stress and accumulation of this mineral in animal tissues and effects of Li on the intestinal microbial community of swines. Our results showed that PLO 02 is more sensitive to high Li concentrations than PLO 13, in which PLO 13 showed a higher mushroom production rate. A positive correlation was observed between the accumulation of Li and other minerals present in the substrate and the effect of Li on the redox state of the fungus. Bioaccessibility in both fungi was higher in Li-enriched mushrooms compared to medicinal lithium carbonate tablet, and these mushrooms can co-accumulate minerals and increase oxidative activity. In the *in vivo* experiment in pigs, Li-enriched *P. djamor* mushrooms was a more bioavailable source of Li than Li_2CO_3, and the Li-enriched mushrooms improved the effects of oxidative enzymes, and in some tissues, swines feed with Li-enriched mushrooms showed less oxidative damage when compared to their corresponding dosages in the form of Li_2CO_3. Through the analysis of the data from the sequencing of the intestinal samples, we verified that Li influences the alpha diversity indices, showing differences in the Simpson, Shannon and Chao-1 indices in the colon and Chao-1 in the feces, depending on the treatment, particularly those treatments that received lithium compared to treatments that did not receive

any form of lithium. The taxonomic grouping of amplicon sequence variants (ASVs) showed that the taxa with the highest relative abundance can vary depend om the tissus, if ileum, colon or feces, with a predominance of the phyla *Firmicutes*, *Bacteroidota* and *Proteobacteria* in treatments that received lithium. Groups of microorganisms important for animal health, such as *Lactobacillus*, *Ruminococcaceae*, *Enterorhabdus*, *Muribaculaceae* and *Coprococcus*, had their relative abundance increased in animals that received Li or Li-enriched mushroom. Mineral bioaccumulating fungi, such as *P. djamor* and *P. ostreatus*, have the potential to be used in human and animal health, because in addition to increasing the availability of Li, it decreases the toxic effects in individuals who make its therapeutic use and increasing populations of important microorganisms that maintain intestinal homeostasis.

Keywords: *Pleurotus* spp. Oxidative stress. Lithium carbonate. Metataxonomy. Organic acids.

SUMÁRIO

INTRODUÇÃO GERAL

Os transtornos depressivos atingem cerca de 5 % da população (aproximadamente 386 milhões de pessoas) e 4 % dos indivíduos correm o risco de desenvolver o transtorno afetivo bipolar durante a vida (Can et al. 2014). O TAB é uma doença caracterizada por alterações episódicas graves de humor no qual os indivíduos acometidos com essa enfermidade têm de 10 a 20 % de chance de cometer suicídio (Ketter 2010; Oruch et al. 2014). O Li é o principal medicamento utilizado para o tratamento do transtorno afetivo bipolar (TAB). Também é usado na prevenção do suicídio e na depressão unipolar (Vosahlikova and Svoboda 2016). Pesquisas têm demonstrado que o Li tem potencial para ser usado no tratamento de uma série de condições neurodegenustivas, como Alzheimer, Parkinson e doença de Huntington (Chiu et al. 2011; Kerr et al. 2018; Lieu et al. 2014; Sarkar et al. 2008) e no tratamento de câncer (Cammarota et al. 2020).

O lítio (Li) é um metal de ocorrência natural na crosta terrestre (0,0017 %), apresentando número atômico 3, massa atômica de 6,941, (Schrauzer, 2002; Shorter, 2009). Na natureza pode ser encontrado na forma de dois isótopos estáveis: ^{6}Li e ^{7}Li, sendo o último o mais abundante e ambas as formas nunca são encontradas livremente, mas em compostos de formas iônicas (Oruch et al. 2014). Esse metal é usado industrialmente na produção de baterias, ar condicionado, produção de cerâmica, reatores atômicos e de fusão (Léonard et al. 1995; Bonino et al. 2011).

O lítio foi descoberto em 1817 pelo químico sueco Johan August Arfvedson no mineral petalita, sendo encontrado também, em quantidades traços em quase todas as rochas. Dessa forma essas rochas sofrem o processo de intemperismo, no qual o Li é mobilizado e transportado para os solos onde poderá ser absorvido pelas plantas e posteriormente entrar na cadeia alimentar (Verspohl, 2006; Robinson et al. 2018).

O lítio também pode ser encontrado, em quantidades traços, nos corpos de água doce e salgada em concentrações entre 1 e 10 µg L^{-1} e 0,18 µg L^{-1}, respectivamente (Schrauzer, 2002; Robinson et al. 2018). As concentrações de Li tanto no solo como na água podem variar de região para região, dependendo do material de origem (Szklarska and Rzymski, 2019). O Li é obtido na alimentação através do consumo de grãos, vegetais, proteínas e algumas águas minerais (Długaszek et al. 2012; Robinson et al. 2018).

Apesar do Li ainda não ser considerado um micronutriente essencial para os seres vivos, ele exerce uma série de funções no organismo e é uma das drogas mais utilizadas no tratamento

de doenças psiquiátricas. A Agência de Proteção Ambiental dos EUA (EPA) e alguns autores recomendam uma dose diária de aproximadamente 1 mg para um adulto de 70 kg (Schrauzer 2002).

Os sais de Li são as formas mais utilizadas no tratamento psiquiátrico, dentre os quais podemos citar o carbonato de lítio (Li_2CO_3), cloreto de lítio (LiCl), citrato de lítio ($Li_3C_6H_5O_7$), sulfato de lítio (Li_2SO_4), oratato de lítio ($LiC_5H_3N_2O_4 \cdot H_2O$) e aspartato de lítio ($C_4H_5Li_2NO_4$), sendo o primeiro o mais usado (Oruch et al. 2014). Entretanto, a administração desses sais, principalmente na forma de carbonato, pode gerar efeitos colaterais como náusea, diarreia, micção e sede excessiva, tremor das mãos, ganho de peso, comprometimento cognitivo, disfunção sexual, problemas dermatológicos, disfunções renais e tireoidianas (Gitlin, 2016). O mesmo autor ainda ressalta que esses efeitos colaterais são a principal causa da baixa adesão dos pacientes ao tratamento com lítio e que esses efeitos podem variar de intensidade de indivíduo para indivíduo.

Ao Li é absorvido totalmente no intestino delgado e distribuído para todo o corpo, com algumas diferenças de concentração nos tecidos, plasma e cérebro (Goldstein and Mascitelli 2016). O lítio é excretado na urina e em menor quantidade nas fezes (2 a 3 %) e suor (Jinhua et al. 2019)

O Li é um cátion semelhante ao sódio, potássio, cálcio e magnésio, competindo com estes íons por sítios de ligação em biomoléculas. Um exemplo é a substituição do magnésio por lítio em enzimas dependentes de magnésio que causa a redução da estabilidade estrutural da enzima diminuindo sua atividade (Shahzad et al., 2017). Como o magnésio é cofator de mais de 300 reações enzimáticas os efeitos podem ser diversos (Vosahlikova and Svoboda 2016).

A toxicidade do Li vai depender da espécie atingida e do tempo de exposição ao Li (Shahzad et al. 2017). A concentração terapêutica sérica de Li em humanos está na faixa de 0,5 a 1 mmol L^{-1}. Sinais de toxicidade leve são observados na faixa de 1,8 a 2,5 mmol L^{-1}, e valores maiores que 2,5 mmol L^{-1} podem levar a uma toxicidade severa (Oruch et al. 2014; Won and Kim 2017). Devemos ressaltar que a toxicidade do Li é causada principalmente devido à sua ingestão frequente no tratamento de alguma doença maníaco-depressiva (Chan et al. 2000).

Embora altamente eficaz na redução dos sintomas do transtorno bipolar, quando em doses terapêuticas, após o tratamento prolongado o lítio pode induzir distúrbios estruturais e funcionais no fígado e rins (Ossani et al., 2019). Há divergências entre estudos que apontam que o Li pode agir como um protetor ao dano oxidativo, embora poucos trabalhos tenham demonstrado um efeito protetor em algum tecido específico, e estudos que apontam um

aumento do dano oxidativo quando o Li está presente no organismo (Toplan et al., 2016; Prokopieva et al. 2019).

As comunidades bacterianas intestinais estão relacionadas ao funcionamento saudável do hospedeiro e evidências apontam que essas bactérias podem estar associadas com o humor e comportamento. Indivíduos com transtorno bipolar apresentam índices maiores de doenças gastrointestinais que podem estar relacionadas à microbiota intestinal (Flowers et al. 2020). Em um estudo com indivíduos bipolares e saudáveis, os autores encontraram diferenças significativas nas comunidades microbianas intestinais (Flowers et al. 2020). Já sobre a relação entre o uso de lítio e a microbiota intestinal há apenas um trabalho, até o momento, relatando que houve um aumento em algumas populações de microrganismos evidenciando que a depender da dosagem e tipo de sal usado as comunidades microbianas podem sofrer mudanças em sua composição (Cussotto et al. 2019).

Devido às várias propriedades medicinais do Li, vem-se despertando o interesse da inclusão desse elemento na alimentação humana, por meio do enriquecimento de alimentos com esse elemento (Hajek and W. Weiner 2016). Fungos produtores de cogumelos vêm sendo estudados com esse propósito, graças a sua capacidade de acumular alguns metais em seus micélios incluindo o Li (de Assunção et al. 2012; Nunes et al. 2014; Mleczek et al. 2017).

Pleurotus spp. possui muitas espécies que produzem cogumelos comestíveis com alto valor nutricional, possuindo diversas propriedades terapêuticas e aplicações biotecnológicas (Nunes et al. 2014). Espécies deste gênero crescem na maioria das madeiras, em subprodutos de madeira (serradura, papel, lodo de polpa), palhas de cereais, espigas de milho, bagaço de cana-de-açúcar, resíduos de café (café, cascos, talos e folhas), cascas de algodão, polpa de soja e em muitos outros materiais (Nunes et al. 2014; Bitew and Mandefro, 2018). Diferentes espécies de fungos produtores de cogumelos podem ser utilizados na produção de alimentos enriquecidos com minerais importantes à saúde como ferro (Scheid et al. 2020), zinco (Umeo et al., 2020), selênio (da Silva et al. 2010) e lítio (de Souza Lopes et al. 2022). Esses trabalhos têm mostrado que os minerais presentes nos cogumelos enriquecidos estão mais bioacessíveis quando comparado às suas formas inorgânicas. Selênio presente em cogumelos enriquecidos com esse mineral estavam mais biodisponíveis no plasma sanguíneo de ratos quando comparados ao grupo que recebeu a forma inorgânica selenito de sódio (da silva et al. 2010). A bioacessibilidade é a quantidade de um nutriente ingerido que fica disponível para absorção no intestino após a digestão. A biodisponibilidade refere-se à fração de um nutriente ingerido que

atinge a circulação sistêmica e os locais específicos onde pode exercer sua ação biológica (Sharidi et al. 2018).

Fungos do gênero Pleurotus são capazes de crescer na presença de diferentes fontes de Li, como o acetato, sulfato, cloreto, hidróxido e carbonato, bioacumulando alguma dessas formas (Nunes et al. 2014; Faria et al. 2018) e acumular esse elemento no cogumelo quando esse elemento está no substrato (de Souza Lopes et al. 2022). Entretanto, não se sabe se esse elemento sofre alguma transformação ou é incorporado em alguma molécula orgânica. Lítio presente em cogumelos enriquecidos de *P. ostreatus* e *Pleurotus djamor* eram mais bioacessível do que o mesmo elemento no medicamento psiquiátrico contendo carbonato de lítio. Porém, o estudo *in vitro* não utiliza todos os fatores fisiológicos envolvidos na absorção e utilização de nutrientes havendo necessidade de estudos *in vivo* para análise de todos esses fatores. Diante do exposto a hipótese do nosso trabalho é que *Pleurotus* spp. pode acumular altas dosagens de Li em seus cogumelos e que esse mineral pode influenciar o status redox, o co-acúmulo de outros minerais, além do cogumelo enriquecido ser uma fonte de Li mais bioacessível que o medicamento a base de carbonato de lítio. Acreditamos também que o cogumelo enriquecido é uma fonte de lítio mais biodisponível menos danosa aos tecidos de leitões tratados com diferentes fontes e formas de Li, além de ter influencia na composição da comunidade microbiona intestinal desses animais.

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CAPÍTULO 1

Bioaccessibility, oxidizing activity and co-accumulation of minerals in Li-enriched mushrooms

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ABSTRACT

Mushrooms present high potential to be used as a source of lithium (Li), but bioaccumulation of Li and other minerals, redox status and bioavailability are specific to each species, depending on the dosage and type of salt used. We investigated the bioaccessibility, oxidizing activity and co-accumulation of minerals in Li-enriched mushrooms in *Pleurotus ostreatus* (PLO 02) and *Pleurotus djamor* (PLO 13). Mushrooms were produced in coffee husks enriched with lithium chloride or lithium carbonate at different concentrations. It was found that PLO 02 is more sensitive to high concentrations of Li than PLO 13, which presented higher mushroom production rate. It was observed a positive correlation between Li accumulation and other minerals present in the substrate and the effect of Li on fungus redox status. Bioaccessibility in both fungi was higher in the Li-enriched mushrooms when compared to the medicinal lithium carbonate tablet, and these mushrooms can co-accumulate minerals and increase oxidative activity. It confirms that Li-enriched mushrooms can be an alternative and healthy source of Li.

KEYWORDS: *Pleurotus*, mushroom, lithium, oxidative stress, bioaccessibility.

INTRODUCTION

Mushrooms belonging to the genus *Pleurotus* spp. are among the most consumed in the world (Sekan, Myronycheva, Karlsson, Gryganskyi, & Blume, 2019). They produce bioactive compounds with antioxidant, antiviral, antibacterial, immunostimulatory and antitumor properties, among other benefits (Sekan, Myronycheva, Karlsson, Gryganskyi, & Blume, 2019). They can also accumulate metals in their mycelia and fruiting bodies and can be used in bioremediation of contaminated environments (Singha, Singhb, & Mishraa, 2020). This is an interesting feature to be explored since we can enrich the mushrooms of these organisms with elements such as zinc, iron, selenium, copper and lithium (de Assunção et al. 2012; Faria et al. 2018; da Silva et al. 2019; Umeo et al. 2020) to be a source of these minerals in human nutrition. This resource gives us the opportunity to research mushrooms as a raw material for the formulation of products that can fight malnutrition and hidden hunger (Adedokun at al. 2016).

Lithium is an element found naturally in the Earth's crust with an abundance of 0.0017%. Its distribution and concentration vary from region to region (Kavanagh, Keohane, Garcia Cabellos, Lloyd & Cleary, 2018). It is used in pharmaceutical and medicinal industries, among others. In their salt forms, such as lithium carbonate (Li_2CO_3) and chloride (LiCl), they have been used for over a century to treat psychiatric illnesses, such as bipolar disorder and schizophrenia (Szklarska and Rzymski, 2019; Voica & Iordach, 2020). At low concentrations, it has mood-stabilizing properties, and 1 mg of Li per day is the recommended dose for a 70 kg adult. Other studies have shown that, in cities where low concentrations of Li are added to drinking water, the incidence of dementia-related illnesses, homicides and suicides decreased (Kessing, et al. 2017). In addition, depending on the dose, Li may have an effect on oxidative status (Toplan et al. 2016), antitumor activity (Taskaeva et al. 2020) and the prevention and treatment of Alzheimer's disease (Hampel et al. 2019).

Little is known about the effect of different sources and concentrations of lithium on the co-accumulation of other nutrients, accessibility and oxidizing activity in mushrooms. Another gap is whether Li undergoes any transformation or binds to any fungal structure. The enrichment of mushrooms with lithium can be an alternative to make this mineral more available for human diet and introduce it into regions where this element is scarce, in addition to being biologically more accessible in mushroom than in medicines (Li_2CO_3) (de Assunção et al. 2012). It is known that some species of *Pleurotus ostreatus* (de Assunção et al. 2012) and *Pleurotus djamor* (Nunes, Cardoso, Luz, & Kasuya, 2015) accumulate high concentrations of Li in their mushrooms and their mycelia, respectively. For *Pleurotus djamor* there are no studies

demonstrating the influence of Li on mushroom production. Therefore, our objective is to evaluate the mushroom productivity in these two fungal species in increasing dosages of LiCl and Li_2CO_3 and consequently the accumulation of this mineral, so that the mushrooms can be used as an alternative source of Li in therapeutic treatments for psychiatric illnesses. We also believe that Li accumulation influences the absorption of other minerals and the redox status of these fungi, in addition to the fact that Li-enriched mushrooms are a more bioaccessible / bioavailable source of Li.

MATERIAL AND METHODS

Fungal, inoculum production and enrichment

Pleurotus ostreatus (PLO 02) and *Pleurotus djamor* (PLO 13) were grown in Petri dishes containing 20 mL of potato-dextrose-agar culture medium, with incubation at 25 ± 1 °C. After 7 days, ¼ of the agar medium colonized by the fungus was transferred to 280 mL pots containing 130 g of sorghum grains which had been previously cooked for 40 min and autoclaved at 127 ºC, for 1 h. After 7 days, of incubation at room temperature, the inoculum was ready to be used.

Coffee husk was used as substrate to produce mushrooms, after being cooked for 2 h and centrifuged at 1500 x g for 1 min. Then, 1 kg of this substrate was placed in a polypropylene bag and autoclaved for 1 h, at 121 ºC. Next, each isolate was inoculated in the substrate with 50 mL of different concentrations of lithium (0.8; 1.6; 2.5; 3.3; 4; 4.9; 5.7; 6.5 g L^{-1}) or (0.2; 0.38; 0.56; 0.75; 0.94; 1.13; 1.32; 1.40 g L^{-1}) in its chloride and carbonate salt form (SIGMA ALDRICH), respectively and incubated at 25 ºC for about 30 days. After the incubation period, the packages were transferred to a fruiting room with controlled temperature and humidity of 20 °C and 80%, respectively. After the mushrooms were harvested, weighed, dehydrated and ground.

Biological Efficiency (EB)

Biological efficiency was calculated according to the methodology of Wang, Sakoda & Suzuki, (2001).

Lithium and other mineral content

Samples of 300 mg of ground dried mushrooms were subjected to digestion with a mixture of nitric acid (SIGMA ALDRICH) and perchloric acid (3:1, v:v) (SIGMA ALDRICH)

at 200 °C for 2 h and the levels of Li, Ca, Cu, Fe, Mg, Mn, K and Zn were determined using an atomic emission spectrometry (FAAS) (Tedesco, Gianello, Bissani, Bohnen, & Volkweiss, 1995)

Oxidizing activity and oxidative stress

Sample preparation

Samples of 100 mg of mushroom were homogenized in 0.2 mol L^{-1} phosphate buffer (SIGMA ALDRICH), 1 mmol L^{-1} ethylenediaminetetraacetic acid (EDTA) (SIGMA ALDRICH), pH 7.4, using a homogenizer. The homogenates were centrifuged at 15,000 x g for 10 min, at 4 °C, and the supernatants were used for analysis of superoxide dismutase (SOD), catalase (CAT) and malondialdehyde (MDA).

Superoxide dismutase (SOD) activity

SOD activity was determined by the method based on the reduction of superoxide (O^{-2}) and hydrogen peroxide (Dieterich, Bieligk, Beulich, Hasenfuss, & Prestle, 2000). The reaction mix contained 170 µL of potassium phosphate buffer (SIGMA ALDRICH) (0.1 mmol L^{-1}, pH 7.8) and 10 µL of sample. The reaction was started by adding 20 µL of pyrogallol (SIGMA ALDRICH) (0.1 mmol L^{-1}), incubated at room temperature in the absence of light, for 30 min. Absorbance was measured at 570 nm. SOD activity was calculated as 1 U of SOD defined as the amount that inhibited the auto-oxidation rate of pyrogallol by 50 %.

Catalase activity

Catalase activity (CAT) was determined according to Aebi, (1984), using hydrogen peroxide (SIGMA ALDRICH) (20 mmol L^{-1}) as substrate. A sample of 100 µL of hydrogen peroxide was added to 10 µL of sample to start the reaction. After 3 min of reaction, 150 µL of molybdate (SIGMA ALDRICH) (32.4 mmol L^{-1}) was added. An extinction coefficient of ε240 = 0.036 mmol L^{-1} cm was used for the calculations. A unit of CAT activity was defined as the amount of enzyme that breaks down 1 mmol hydrogen peroxide in 1 min (Aebi, 1984).

Malondialdehyde determination

The extent of lipid peroxidation (LPO) was measured using malondialdehyde (MDA), which is the main product of lipid peroxidation (Buege & Aust, 1978). In order to start the reaction, 0.200 µL of the tissue supernatant were homogenized in 400 µL of a solution of

trichloroacetic acid (MERCK MILIPORE) (15 %): thiobarbituric acid (MERCK MILIPORE) (0.375 %): hydrochloric acid (MERCK MILIPORE) (0.6 %). The samples were kept in a water bath, at 90 ºC, for 40 min. Then, the samples were kept on ice for 5 min. Soon after butyl alcohol (SIGMA ALDRICH) (0.6 mL) was added, the solution was vortexed for 2 min and centrifuged for 10 min, at 9000 *g*, and the precipitate was removed. The supernatant was used to measure absorbance at 535 nm. MDA concentration was determined using the standard curve of known concentrations of 1, 1, 3, 3-tetramethoxypropane (TMPO) (SIGMA ALDRICH). The results were expressed in μmol L^{-1} per mg of protein (Kruger, 2009).

In vitro digestion

Lithium accessibility was verified by *in vitro* digestion according to the methodology of Scheid et al. (2020). For this we used 1 mg of enriched mushroom of PLO 02 and PLO 13 grown in dosages 0, 0.8, 2.5, 4.0, 5.7, 6.5 g L^{-1} having as source LiCl (SIGMA ALDRICH) and 0, 0.2, 0.5, 0.75, 1.3, 1.4 g L^{-1} having as source Li_2CO_3 (SIGMA ALDRICH). To compare the bioaccessibility of these mushrooms in relation to the drug used in the treatment of psychiatric illnesses, lithium carbonate medicine was also submitted to *in vitro* digestion. For each enriched mushroom sample we used a mass of lithium carbonate so that both had the same content of this mineral and could be compared for their bioaccessibility.

Statistical analysis

The experiment was conducted in a completely randomized design with 8 concentrations of lithium chloride and 8 concentrations of lithium carbonate and 6 replications. The data were analyzed for variance (ANOVA), Tukey test or regression, at 5 % significance, using the R software system.

RESULTS AND DISCUSSION

Biomass and Biological Efficiency

Mushroom production and biological efficiency (BE) for both isolates of fungi were negatively affected by the concentration of LiCl and Li_2CO_3 in the substrate ($p<0.05$). PLO 02 was more affected by higher concentrations of LiCl than PLO 13. PLO 02 biomass and BE reach almost zero at a concentration of 6.5 g L^{-1} of Li (Fig. 1a and 1c). PLO 13, on the other hand, maintains its biomass of about 130 g per kg of substrate and 60% of BE, even at the highest concentration of LiCl (Fig. 1a and 1c). As for lithium carbonate, both fungi reduced

biomass and BE at higher concentrations (Fig. 1b). Mleczek and collaborators (2017) also observed that the lithium source negatively affected the biomass of fruit bodies of *Pleurotus ostreatus* at high concentrations of Li_2CO_3 (1 mmol L^{-1}), and affected less when cultivated in lithium acetate. In our work, PLO 02 enriched with 1 mmol L^{-1} (0.2 g L^{-1}) of Li, having Li_2CO_3 as a source, provided a biomass of 150 g of mushroom per kg of substrate, which did not differ from the control, that is, this salt at this concentration did not affect the biomass of our isolate (Fig. 1b). The same behavior was observed for PLO 02 grown in LiCl, both for biomass and BE, which demonstrates that these fungi can be used for food biofortification, especially when grown at low concentrations of LiCl and Li_2CO_3 sources.

Few studies have approached the biomass of these fungi at high doses of LiCl or Li_2CO_3. Most studies have focused on the mycelial growth of these fungi in different sources of Li. Nunes, Cardoso, Luz & Kasuya, (2015) examined fungal species and found that the less sensitive species was *P. djamor* cultivated in different sources and at different Li concentrations. In our work, PLO 13, a *P. djamor,* presented tolerance to high doses of Li, which decreased when values above 0.94 g L^{-1} ($p<0.05$) were used.

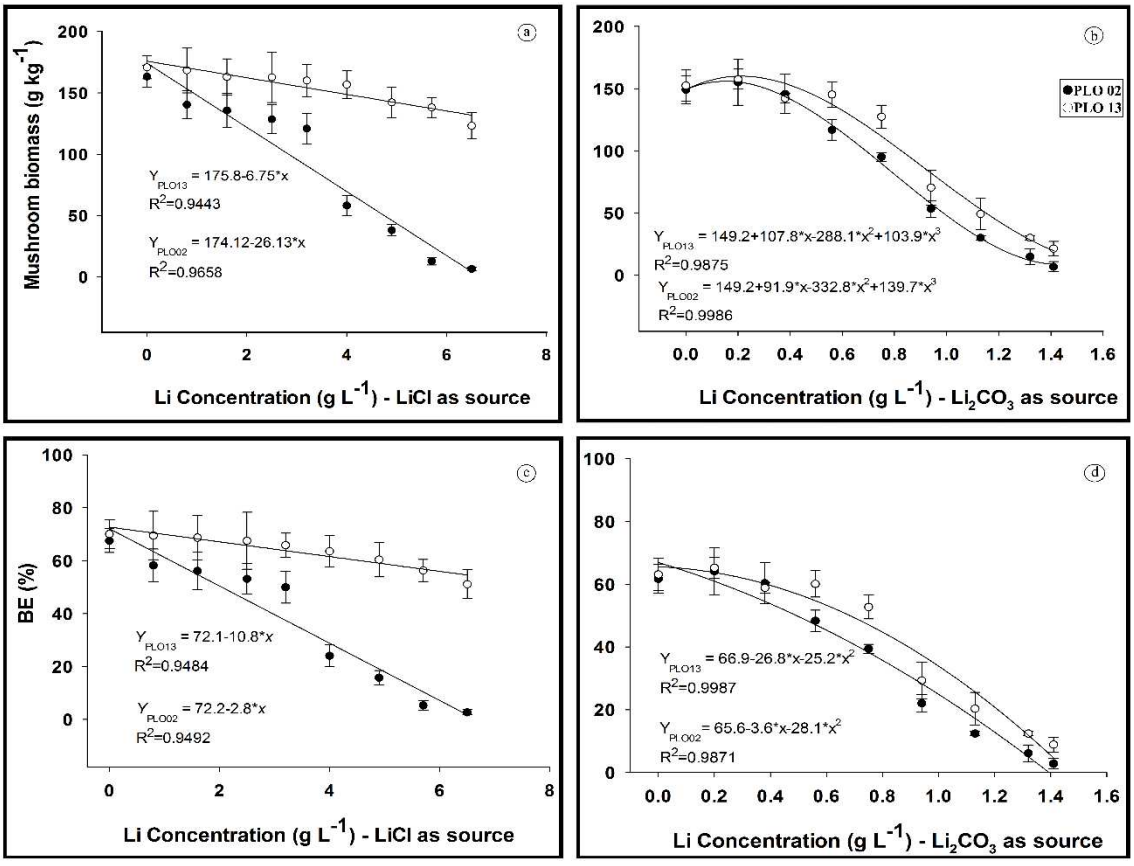

Figure 1. Biomass and biological efficiency (BE) of mushrooms (g kg^{-1}) produced by *Pleourotus ostreatus* (PLO 02) and *Pleurotus djamor* (PLO 13) grown in coffee husks at different concentrations of Li, when grown in a substrate enriched by LiCl (a and c) or Li_2CO_3 (b and d).

Lithium content

Positive correlation was observed between dosages of Li added to the substrate and Li accumulation in mushrooms (Fig. 2a and 2b). In the comparison of the salts added to the substrate, when LiCl was used, higher Li accumulation values were observed in mushrooms for both fungi. PLO 02 accumulated a maximum of 135 and 3 µg g^{-1} of Li, for the fungus enriched with LiCl and Li_2CO_3, respectively (Fig. 2a and 2b). For PLO 13, the values were 460 and 70 µg g^{-1} for the same salts, respectively. When the fungi are compared, it is clearly observed that PLO 13 is more efficient in accumulating Li in their fruiting bodies. When grown on the substrate enriched with LiCl, PLO 13 accumulates more than twice as much Li than PLO 02 (Fig. 2a). In the substrate enriched with Li_2CO_3, PLO 13 accumulated about 30 times more Li than PLO 02.

Both Li salts and fungus species tested affected Li accumulation in mushrooms, which agrees with the data from Mleczek et al., (2017), who observed that, when *Ganoderma lucidum*, *P. eryngii* and *P. ostreatus* were enriched with Li carbonate or Li acetate, at concentrations ranging from 0.25, 0.50, 0.75 to 1 mmol L^{-1}, the maximum Li accumulation was obtained by *P. ostreatus* mushrooms, which was 12 µg g^{-1} in 1 mmol L^{-1} of Li_2CO_3, an accumulated value much higher than that found in our work by PLO 02 (0.67 µg g^{-1}) in the same salt and at the same concentration. These differences in results may be related to the conditions of cultivation and the substrate used. *Pleurotus ostreatus* grown on coffee husks supplemented with 15 mL of a LiCl solution (500 mg L^{-1}) accumulated about 200 µg g^{-1} of Li (de Assunção et a., 2012). They also observed that Li accumulation in the fruiting bodies was related to the increased dose of salt added to the substrate.

A possible explanation for the greater accumulation of Li by both fungi in LiCl compared to Li_2CO_3 is the substrate pH after the addition of the solutions of these salts. In Figure 2, it is observed that the pH variation in LiCl solutions is much smaller compared to the pH variations in Li_2CO_3 solutions (Fig. 2c and 2d). Besides, Li is more absorbed in acidic soils than alkaline soils (Shahzad et al., 2016), probably because soil acidity corresponds to increased Li solubility.

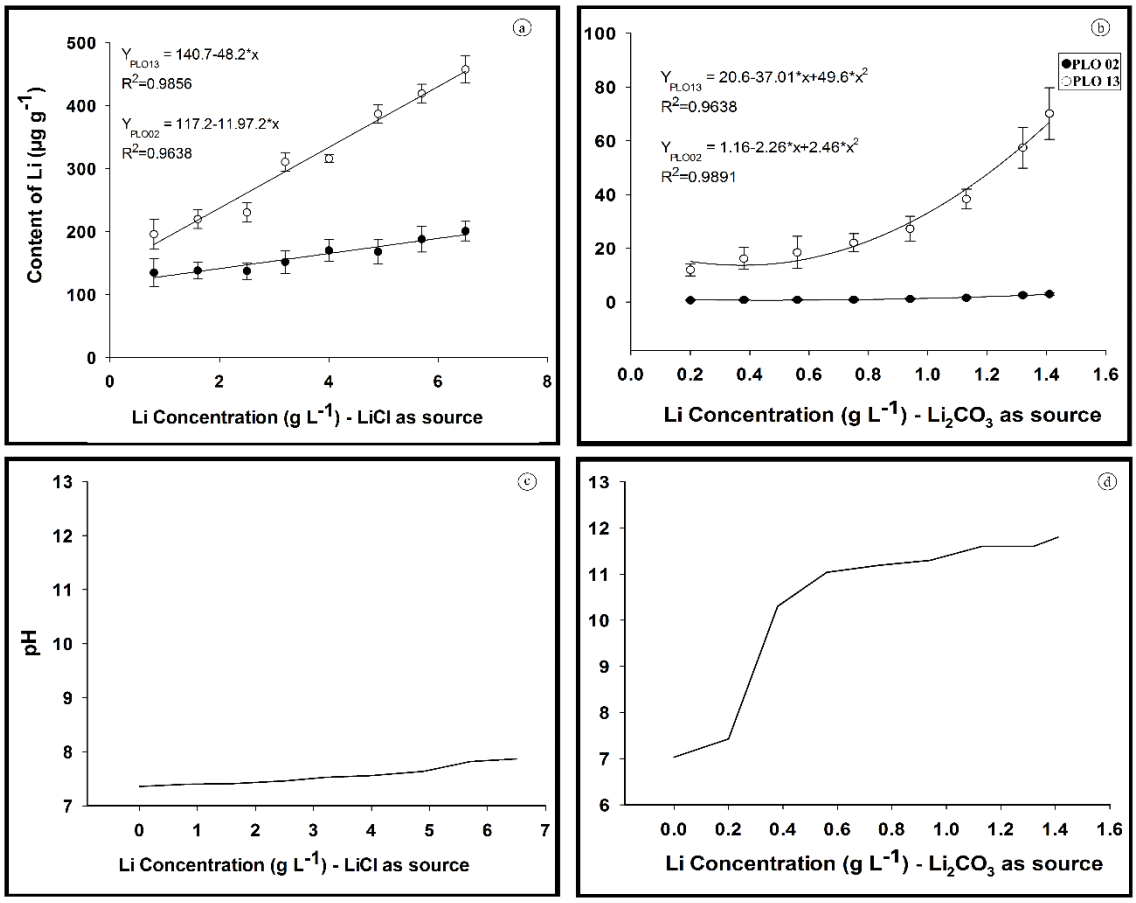

Figure 2. Bioaccumulation of Li in mushrooms of *P. ostreatus* (PLO 02) and *P.djamor* (PLO 13) grown in substrate enriched with LiCl (a) or Li_2CO_3 (b) at different concentrations of Li and pH of the solutions added to the substrate (c and d).

We found that PLO 13 is so far from the fungus that succeeds accumulate the highest levels of Li, especially for LiCl, without losing much in its productivity, compared with studies with other species of fungi (de Assunção et al. 2012; Rzymski et al. 2017; Mleczek et al. 2017). PLO 13 mycelium, which is the fungus that grew the most when this salt was added to the culture medium, is resistant to high doses of LiCl (Nunes, Cardoso, Luz & Kasuya, (2015).

The recommended consumption is 1 mg of Li per day, since this element presents several beneficial effects on health and mood. Thus, the consumption of 2.2 g of PLO 13 dried mushroom per day would provide all the necessary Li. The use of Li in psychiatric disorders requires daily dosages of about 300 to 1200 mg of Li_2CO_3 (56.5-226 mg of Li) of this salt, which can be administered orally up to 3 times a day (Mleczek et al. 2017; Oruch, Elderbi, Khattab, Pryme & Lund, 2014). The consumption of 121 g of dried mushroom of PLO 13 would provide this amount of Li for patients who use lower dosages (300 mg), which can be increased later. Another interesting factor in the use of this mushroom is that the Li present is more

accessible to the body than the medicine (Assunção, et al. 2012). Therefore, lower dosages could be necessary to reach the desired serum levels, thus reducing the side effects caused by the use of these medications (Oruch, Elderbi, Khattab, Pryme & Lund, 2014).

Li accumulation and co-accumulation of other minerals

The most abundant minerals found in the substrate used for the fungus to grow were Cu, K, Fe and Ca, respectively (Table 1). These data agree with other works, in which these minerals are the most abundant in coffee husk (de Assunção et al. 2012; Gemechu, 2020), except for Cu, which was found in greater concentrations in the residue in our work. This residue contains almost all essential minerals for human health that are involved in the regulation of the metabolic and physiological functions of the human body. Therefore, it proves to be a by-product of agricultural activity that can be used in food production (Gemechu, 2020).

Table 1. Mineral composition of the coffee husk substrate compared to literature data.

Mineral composition of coffee husk substrate (mg g^{-1})							
Ca	**Cu**	**Fe**	**Mg**	**Mn**	**K**	**Zn**	**Reference**
8.3	71.1	8.4	0.8	0.062	17.8	0.011	Our work
4.3	*-	1.4	0.8	*-	3.59	0.030	de Assunção et al. 2012
7	18	233	1.5	0.9	22.6	56	Gemechu, 2020

*Not determined

Macrominerals are present in large amounts in living organisms and are required in large amounts in the diet. These, e.g. K, Ca and Mg, should have their intake greater than 100 mg day^{-1} (Spada et al., 2010). The daily requirements of microminerals, e.g. Cu, Fe, Mn and Zn, are lower than 100 mg day^{-1}. These minerals were quantified in PLO 02 and PLO 13 mushrooms, in order to verify if Li could interfere with the absorption of other minerals.

PLO 02 and PLO 13 presented a positive linear correlation between Li increase (LiCl as source) in the substrate and the accumulation of K and Cu ($r>0.90$) (table 2). As for Ca, Fe, Zn and Mn, these minerals co-accumulate up to a certain concentration of Li (LiCl as source) in the substrate and decrease at Li highest concentrations (table 2). For both fungi, only Mg did not have its accumulation altered by the increased concentrations of Li (LiCl as source) added to the substrate (table 2). For all these minerals, when the source of Li was LiCl, PLO 13 presented higher levels of accumulation, compared to PLO 02, except for Zn.

When the source of Li was Li_2CO_3, a positive correlation was observed between Li dosage and the accumulation of K, Ca and Fe ($r>0.90$) for PLO 02, and Cu in PLO 13 ($r>0.90$). As for Zn, Cu and Mn (PLO 02) and K, Ca, Fe, Zn and Mn (PLO 13), an accumulation of these minerals was observed up to a certain dosage of Li used, which decreased at the highest Li

dosages. Again, no relationship was verified between Li dosage and Mg accumulation in any of the fungi.

Table 2. Mineral profile in Li-enriched mushrooms of *Pleurotus ostreatus* (PLO2) and *Pleurotus djamor* (PLO 13), at different concentrations of lithium, using LiCl or Li_2CO_3 as source of Li in coffee husk-based substrate.

Mineral	Fungi	Li (g L^{-1}) (LiCl)										
		0	**0,8**	**1,6**	**2,5**	**3.2**	**4**	**4,9**	**5,7**	**6,5**	**Means**	**R^2**	**Regression Equation**
K (mg g^{-1})	**PLO02**	19.3b	24.7b	28.2b	27.9b	30.5a	29.3a	32.5a	32.4a	32.8a	-	0.961	Y=23.8+3.98**x*-0.34*x^2
	PLO13	22.7b	24.8b	27b	26.7b	26.9b	27b	28a	30.9a	31a	-	0.935	Y=23.7+1.09*x
Ca (mg g^{-1})	**PLO02**	0.37b	1.4a	1.5a	0.93a	0.92a	0.87a	0.62b	0.58b	0.50b	-	0.862	Y= 0.5+0.94*x-0.34*x^2+0.03*x^3
	PLO13	1.4b	3.5a	4.4a	6.9a	5.9a	2.9a	2.4a	2b	1.8b	-	0.903	Y= 1+4.9**x*-1.5*x^2+0.12*x^3
Mg (µ g^{-1})	**PLO02**	1.1	1.3	1.4	1.3	1.2	1.2	1.1	1.2	1	1.2a	*	-
	PLO13	0.98	1.03	1.02	1.06	1.07	1.03	0.96	0.96	1.04	1.0b	*	-
Fe (µ g^{-1})	**PLO02**	95.2b	88.1b	102.3b	158.7a	159.6a	157a	179a	157.1a	148.5a	-	0.905	Y= 77.2+36.10**x*-3.74*x^2
	PLO13	136.5b	136.8b	162.5a	192.2a	210.6a	207.6a	231.8a	273a	259.7a	-	0.975	Y= 130.9+21.6**x*
Zn (µ g^{-1})	**PLO02**	44.4b	45.2b	51a	62a	65a	60a	59.6a	60.5a	60.4a	-	0.917	Y= 42.2+8.9**x*-0.97*x^2
	PLO13	34.8a	63.2b	77.4b	76.4b	76.4b	80.3b	79.9b	71.5b	69.2b	-	0.927	Y= 45.5+20.4**x*-2.6*x^2
Cu (µ g^{-1})	**PLO02**	1.4b	2.3a	7a	9.4a	16.3a	19.7a	19.8a	25a	26a	-	0.976	Y=1.6+1**x*-0.08*x^2
	PLO13	0.72b	0.70b	2.4b	6.8b	9.6a	10.4a	10.3a	10.5a	7.7a	-	0.942	Y=-1.28+4.5**x*-0.45*x^2
Mn (µ g^{-1})	**PLO02**	2.4b	6.8a	9a	13a	8.5a	4.2b	4.2b	3.8b	2.8b	-	0.917	Y=1.9+9.5**x*-3.2*x^2+0.27*x^3
	PLO13	4.8b	7.1a	10.7a	15.1a	12.2a	11a	10.6a	10a	10.3a	-	0.915	Y=3.97+7.4**x*-1.95*x^2+0.15*x^3
		Li (g L^{-1}) (Li_2CO_3)											
		0	**0.2**	**0.38**	**0.56**	**0.75**	**0.9**	**1.1**	**1.3**	**1.4**	**0**	**0.2**	
K (mg g^{-1})	**PLO02**	19.3b	23.2b	24.1b	25.7b	26a	26.6a	28b	28a	28a	-	0.987	Y= 20.11+11.9**x*-4.7*x^2
	PLO13	22.7b	27.8a	28a	28.2a	28.2a	27.9a	27.6a	24.2a	23.7b	-	0.937	Y= 23.70+14.85**x*-10.6*x^2
Ca (mg g^{-1})	**PLO02**	0.37b	0.39b	0.59b	0.80b	0.81b	0.98b	1.15b	1.35a	1.84a	-	0.972	Y= 0.39+0.19**x*+0.49*x^2
	PLO13	1.46a	1.48a	1.48a	1.50a	2.20a	2.20a	1.45a	0.78b	0.78b	-	0.796	Y= 1.19+2.3**x*-2.8*x^2
Mg (µ g^{-1})	**PLO02**	1.1	1.1	1.2	1.2	1.2	1.2	1.2	1.2	1	1.1a	*	-
	PLO13	0.98	1.2	1.2	1.2	1.2	1.1	1.1	0.94	0.97	1.0b	*	-
Fe (µ g^{-1})	**PLO02**	95.2b	95.2b	95.3b	98.6b	106b	114b	121.8a	139.5a	129.8a	-	0.971	Y= 94.11**x*+22.3*x^2;
	PLO13	140.4a	197.5a	186.3a	182.8a	164.9a	164.3a	122.7b	115.7b	114b	-	0.895	Y= 159.8+87.8**x*-91.7*x^2
Zn (µ g^{-1})	**PLO02**	44.5b	55.5a	59.4a	59.2a	59.6a	67.3a	60.5a	60.7a	49.3b	-	0.894	Y= 44.2+46.96**x*-28.6*x^2
	PLO13	34.8b	63.2a	77.4a	76.4a	76.4a	80.3a	79.9a	71.5a	58.4a	-	0.945	Y=40.9+96.2**x*-55.1*x^2
Cu (µ g^{-1})	**PLO02**	1.9b	2.4b	2.4b	3.9a	4a	4.8a	5a	5.2a	4.5a	-	0.971	Y=1.56+4.6**x*-1.6*x^2
	PLO13	0.73b	1.73b	2b	2.6b	3.6b	3.6b	3.8b	5.5a	5.8a	-	0.980	Y=0.96+2.69**x*+0.41*x^2
Mn (µ g^{-1})	**PLO02**	2.4b	6.9a	7.2a	7.9a	9.3a	12.9a	8.5a	8.2a	6.9a	-	0.887	Y=2.6+17.6**x*-10.2*x^2;
	PLO13	4.8b	10.5a	11.4a	10a	9.25a	7.4b	6.23b	4b	3.7b	-	0.903	Y=6.7+12.08**x*-10.6*x^2.

Means with different letters differ between fungi for each mineral, at 5% probability level by Tukey's test. *No correlation between Li dosage in the substrate and the accumulation of this mineral. The slope is equal to zero

The Li is known to replace minerals, such as K, Ca, Mg and Na, in biological systems due to the similarities in size and charge with these elements (Shahzad, Mughal, Tanveer, Gupta, & Abbas, 2017). However, only one paper reports mushroom enrichment and co-accumulation of other minerals (Pankavec et al. 2021). In spinach grown at different concentrations of lithium chloride monohydrate, a continuous increase was verified in the concentration of K content, but Ca decreased in the leaves, as the concentration of Li increased (Bakhat et al. 2019). In *Lolium perenne*, increased Li concentrations reduced Mg uptake (Yalamanchali, 2012). It is believed that the decreased absorption of these two ions at high concentrations of Li occurs because it replaces K, Ca and Mg in plant absorption sites, or inhibits these transporters (Epstein, 1960; Yalamanchali, 2012; Bakhat et al. 2019).

The Zn accumulation in mushrooms did not significantly change with the increased concentration of Li_2CO_3 added to the residue of the fungus cultivation (Li concentration ranging from 0 to 0.10 g per kg of substrate), with a maximum accumulation of 42 µg g^{-1} for both control and treatments (Pankavec et al. 2021). As for Cu, the same study reported increased absorption of this metal as the concentration of Li_2CO_3 increased in the residue where the fungus grew, reaching 0.026 mg g^{-1} compared to its control, which accumulated 0.012 mg g^{-1}, thus corroborating our results (Table 2). In the aforementioned work, Mn levels in mushrooms decreased in treatments with Li, compared to its control with maximum accumulations of 3.6 and 5.5 µg g^{-1}, respectively.

As observed, the Li-enrichment of mushrooms under these conditions and substrate led to a co-accumulation of essential minerals for human health, that is, in addition to being able to grow and be enriched with Li in agro-industrial residues, PLO 02 and PLO 13 co- accumulate essential minerals in their mycelium, without adding them to substrates, which makes them excellent food alternatives rich in compounds and essential elements for health.

Oxidizing activity and oxidative stress

The SOD activity was higher in PLO 02 mushrooms, when compared to PLO 13. Its activity increased as the concentration of both Li salts increased, while decreased levels were found at the highest concentrations of Li_2CO_3 (Fig. 3a and 3b). The highest levels of SOD activity in PLO 02 were 12.85 and 7.2 U mL^{-1}, respectively, in LiCl and Li_2CO_3. In PLO 13, the highest levels were 7.8 and 7.3 U mL^{-1}, respectively, in LiCl and Li_2CO_3.

CAT presented a behavior similar to that of SOD, but PLO 13 presented higher activity levels than PLO 02 (Fig. 3c and 3d). The highest levels of CAT activity in PLO 02 were 155

and 57 U mL^{-1}, respectively, in LiCl and Li_2CO_3. In PLO 13, the highest levels were 210 and 179 U mL^{-1}, respectively, in LiCl and Li_2CO_3.

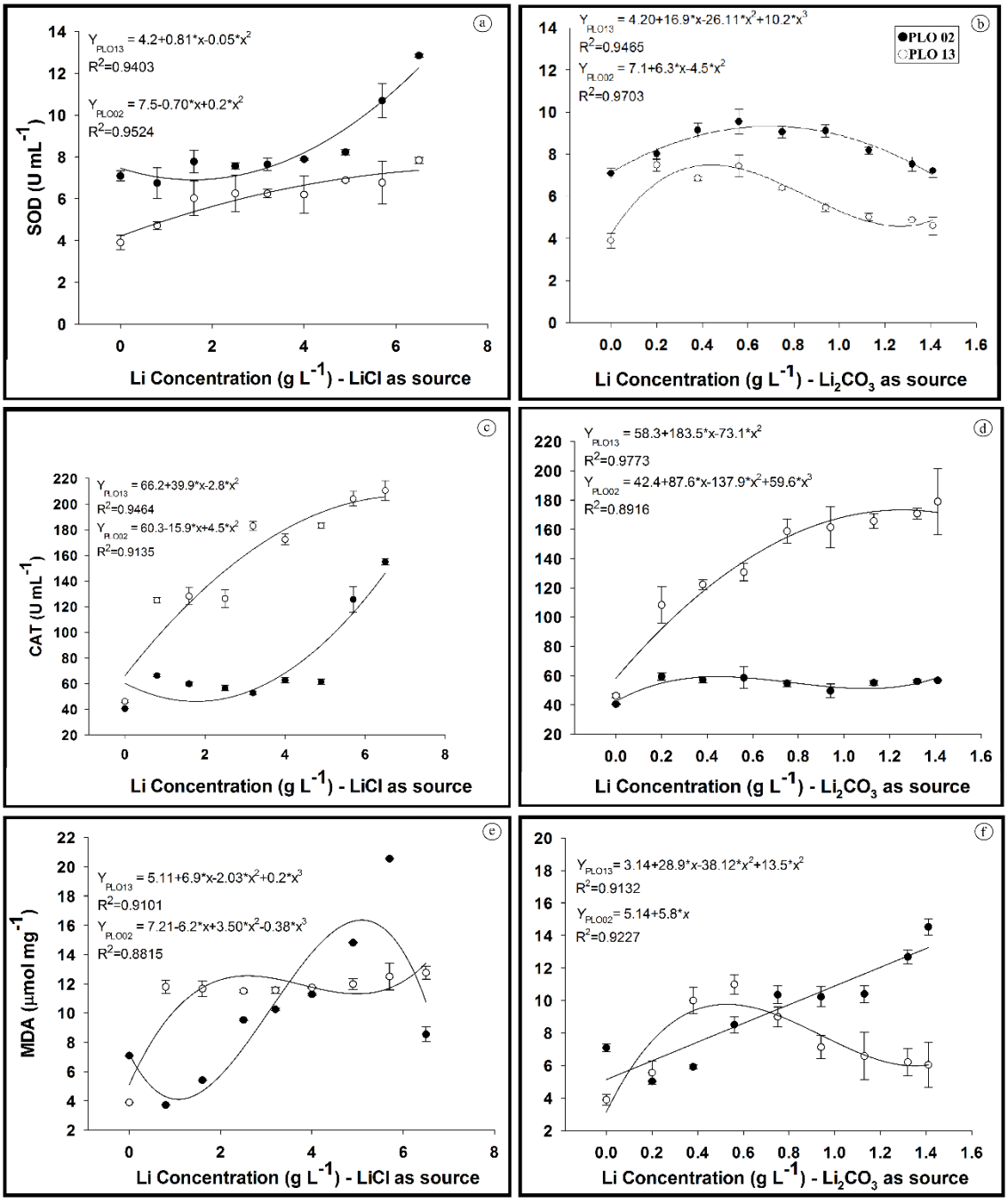

Figure 3. Activity of catalase (CAT) and superoxide dismutase (SOD) enzymes and oxidative stress marker (MDA) in *Pleurotus ostreatus* (PLO 02) and *Pleurotus djamor* (PLO 13) mushrooms grown on coffee husks based substrate, with different concentrations of Li, such as LiCl or Li_2CO_3.

MDA values in both mushrooms are lower in the controls. They increase as the concentrations of Li salts increase, and decrease at the highest concentrations of LiCl and Li_2CO_3, respectively, in PLO 02 and PLO 13. PLO 02 presented higher levels of MDA as the

concentration of Li salts increased, and the highest values in LiCl were obtained with about 20 µmol mg^{-1}. On the other hand, in the same source of Li, PLO 13 presented little change in MDA levels, with about 3.9 and 12.7 µmol mg^{-1}, in that order, for the control and mushroom cultivated in 6.5 g L^{-1} of LiCl. This may indicate that Li salts can be harmful to membrane lipids at the highest concentrations of these salts, which is evidenced by the increased oxidative stress as the concentrations of these salts to the substrate in which the fungus is cultivated are increased.

In *P. ostreatus,* it was verified that the increased concentration of LiCl in the substrate was related to the reduced oxidant activity in this fungus, that is, LiCl causes oxidative stress at higher doses (Vieira et al. 2013). This result is in agreement with the findings of our work, in which we verified increased levels of the oxidative stress marker as we increased the concentration of both Li salts. As observed, PLO 13 grown in LiCl presented lower oxidative damage than PLO 02. We can relate this to the biological efficiency of PLO 13, which was much higher than that of PLO 02 in this salt. In order to produce mushrooms with high concentrations of Li, in which there is less oxidative damage in the fungi, we recommend the cultivation of PLO 13 in the highest dosage of Li in the form of chloride (6.5 g L^{-1}) since the levels of MDA are maintained with few changes in the different concentrations of this element added to the substrate, which would also ensure greater productivity (figure 1a).

Lithium Bioaccessibility

The percentage of Li obtained after *in vitro* digestion increased as the dose of Li added to the substrate increased, which consequently increased the amount of Li present in the fruiting bodies for both fungi grown in LiCl. Through in vitro digestion, we were not able to quantify the bioavailability of the mineral, but we can measure accessibility, which is a factor that affects bioavailability.

PLO 02 presented statistically greater accessibility than the medicine (Li_2CO_3), when grown in both Li salts (Table 3).

Mushrooms of PLO 02 cultivated in LiCl had their bioaccessibility increased from 74 to 78 % at the dosages of 0.8 and 6.5 g L^{-1}, respectively. The 1.94 mg Li_2CO_3 medicine (which contains the same dose of Li as the mushrooms produced in 6.5 g L^{-1} of LiCl) presented maximum accessibility of 74% (Table 3).

Table 3. *In vitro* accessibility of Li-enriched *Pleurotus ostreatus* (PLO 02) mushroom grown on substrate with different doses of LiCl or Li_2CO_3 and the medicine (Li_2CO_3 tablet) based on gastrointestinal digestion, using the same concentration of Li.

PLO 02 grown in LiCl			
LiCl dose (g L^{-1}) [a]	**Li content in the mushroom[b] (µ g^{-1})**	**Lithium Accessibility (%)**	
		Mushroom	**Li_2CO_3 medicine**
0	0	0	0
0.8	134.6	74a	64b
2.5	137.1	73a	63b
4	169.8	75a	71b
5.7	187.9	76a	73b
6.5	200.6	78a	74b
Means	**-**	-	-
R^2	**-**	0.9936	0.9981
Regression Equation	**-**	$Y= 0.82-0.034*x+0.0075*x^2$	$Y = 0.74-0.0098*x+0.0024*x^2$

PLO 02 grown in Li_2CO_3			
Dose Li_2CO_3(g L^{-1}) [a]	**Li content in the mushroom[b] (µ g^{-1})**	**Lithium Accessibility (%)**	
		Mushroom	**Li_2CO_3 medicine**
0	0	0	0
0.2	0.67	71	69
0.56	0.85	71	70
0.75	1.1	72	71
1.3	2.53	72	70
1.4	2.94	73	70
Means	-	72a	70b
R^2	-	-	-
Regression Equation	-	-	-

Both the Li source and the dosage affected bioaccessibility (Table 3). Li-enriched PLO 02 mushrooms using LiCl reached the maximum bioaccessibility of 78% against 74% of the Li_2CO_3 medicine with the same Li content as the mushrooms grown at this dosage (6.5 g L^{-1}). When cultivated in Li_2CO_3, only the Li source used in digestion affected bioaccessibility. In other words, accessibility was not affected by the Li concentrations used to cultivate the fungi (Table 3).

The bioaccessibility of Li in PLO 13 grown with LiCl reached 92 % at the highest Li dose (Table 4). This value is statistically higher when compared to the same Li dose present in the Li_2CO_3 medicine (Table 4). In this case, both the source of Li used in digestion and the dosages used can affect the accessibility of this mineral (Table 4). The accessibility of this mineral is greater in the mushroom enriched with Li_2CO_3 when compared to the Li_2CO_3 medicine, even without being affected by the dosages (Table 4).

Table 4. *In vitro* accessibility of lithium in Li-enriched *Pleurotus djamor* (PLO 13) mushroom (with LiCl or Li_2CO_3) and medicine (Li_2CO_3 tablet) based on gastrointestinal digestion, using the same concentration of Li.

PLO 13 grown in LiCl			
LiCl dose (g L^{-1}) [a]	**Li content in the mushroom[b] (µ g^{-1})**	**Lithium Accessibility (%)**	
		Mushroom	**Li_2CO_3 medicine**
0	0	0	0
0.8	195.7	80a	66b
2.5	230.3	80a	69b
4	315.6	80a	74b
5.7	418.9	87a	77b
6.5	457.5	92a	78b
Means	**-**	-	-
R^2	**-**	0.9981	0.9910
Regression Equation	**-**	$Y= 0.82-0.034*x+0.0075*x^2$	$Y = 0.64+0.02*x-0.0005*x^2$

PLO 13 grown in Li_2CO_3			
Dose Li_2CO_3 (g L^{-1})[a]	**Li content in the mushroom[b] (µ g^{-1})**	**Lithium Accessibility (%)**	
		Mushroom	**Li_2CO_3 medicine**
0	0	0	0
0.2	11.98	67	66
0.56	18.44	69	70
0.75	27.24	72	70
1.3	57.45	70	70
1.4	70.14	72	72
Means	-	71a	70b
R^2	-	-	-
Regression Equation	-	-	-

Means with different superscript letters differ at 5% probability level by Tukey's test. [a]Dose used to enrich the substrate on which the fungus grew. [b]Li content per gram of mushroom.

De Assunção et al. (2012) presented a result similar to ours. Their study reported that PLO 02 mushrooms grown in coffee husks and enriched with LiCl were a more accessible source of Li, compared to Li_2CO_3 tablet. This is the first work that demonstrates that Li bioaccessibility in mushrooms depends on the fungus species and the type and concentration of salt used in their cultivation. We demonstrate that bioaccessibility increased with dose when LiCl was used for fungal cultivation and that, in both salts, the bioaccessibility is greater in mushrooms compared to the drug commonly used in the treatment of psychiatric illnesses.

CONCLUSIONS

The fungi used in this study presented a positive relationship between the dose added to the substrate and Li accumulation in their mushrooms. PLO 13 is Li-accumulator with no loss in productivity. There is a co-accumulation of some essential minerals when the fungus is cultivated with Li and this mineral affects the oxidative state of these fungi. PLO 13 presented lower levels of MDA, which reflects in less oxidative stress. Li accessibility presents a positive

relationship with the dosage used. Both fungi can be used as an alternative source of the daily needs of this mineral. For higher dosages (therapeutic levels), PLO 13 may be a good option, as it accumulates the highest levels of Li found in the literature so far.

Acknowledgments

The authors are grateful to the Cordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) for financial their support.

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CAPÍTULO 2

Bioavailability of Li-enriched mushrooms and protection against oxidative stress in swines: First study *in vivo*

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ABSTRACT

Background: Edible mushroom are capable of bioaccumulating minerals in their mycelia and fruiting bodies. Furthermore, minerals in mushrooms are more bioaccessible than in salts which include lithium (Li). Therefore, the present work aimed to verify, in piglets, the bioavailability of Li-enriched mushroom and the relationship between the ingestion of lithium and the oxidative state of tissues (blood, brain, liver, and kidney).

Methods: Piglets 28-30 days-old were feed for five days and the Li quantification and cortisol were performed by atomic emission and chemiluminescence, respectively. The antioxidant enzymes and the stress marker were analyzed by spectrophotometry.

Results: The highest levels of cortisol were found in the highest dosage of Li and in the control. Based on the piglets' daily serum levels, we found that Li-enriched mushrooms were a more bioavailable source of Li to the body when compared to Li_2CO_3, and ingestion of Li-enriched mushrooms improved the effects of oxidative enzymes and, in some tissues, presented less oxidative damage when compared to its corresponding dosage as Li_2CO_3.

Limitations: Our limitation may have been the period of the experiment since the effects of lithium in the body are greater with longer use.

Conclusions: These results demonstrate the potential to use Li-enriched *Pleurotus djamor* as an alternative source of Li that is more bioavailable and present protective effects against oxidative stress.

KEYWORDS: *Pleurotus djamor*, cortisol, superoxide dismutase, catalase, malondialdehyde.

INTRODUCTION

Edible mushrooms are capable of bioaccumulating some minerals in their mycelium and fruiting body, which enables them to supply the deficiency of these nutrients (da Silva et al., 2012; Vieira et al., 2013; Hu et al., 2020). In addition to being a highly healthy food source, fungi can be managed to make some minerals more bioavailable, which is the case of selenium (Hu et al., 2020) and more bioaccessible, as demonstrated to lithium (de Assunção et al., 2012). Lithium (Li) is one of the minerals used in mushroom enrichment (de Assunção et al., 2012; Vieira et al., 2013; Faria et al., 2019). *Pleurotus* spp is capable of growing in the presence of different sources of Li, such as acetate, sulfate, chloride, hydroxide and carbonate, and bioaccumulate it in the mushroom (Faria et al., 2019). However, it is not known whether this element undergoes any transformation or if it is incorporated into some organic molecule. De Assunção and collaborators (2012) demonstrated *in vitro* that the Li-enriched mushrooms of *Pleurotus ostreatus* were more accessible than the same element in the psychiatric medication containing lithium carbonate. However, the *in vitro* study does not use all the physiological factors involved in the absorption and use of nutrients, and *in vivo* studies are necessary for the analysis of all these factors.

Although Li is not yet considered an essential micronutrient for living beings, it performs a number of functions in the body and is one of the most used drugs in the treatment of psychiatric diseases, especially Li salts, including lithium carbonate (Li_2CO_3), lithium chloride (LiCl), lithium citrate ($Li_3C_6H_5O_7$), lithium sulfate (Li_2SO_4), lithium orate ($LiC_5H_3N_2O_4 \cdot H_2O$) and lithium aspartate ($C_4H_5Li_2NO_4$), which is the most commonly used (Oruch et al., 2014). However, the administration of these salts, mainly in the form of carbonate, can genuste side effects, such as nausea, diarrhea, urination and excessive thirst, hand tremor, weight gain, cognitive impairment, sexual dysfunction, dermatological problems, kidney and thyroid dysfunctions (Gitlin., 2016). These authors also point out that these side effects are the

main cause of low adherence of patients to lithium treatment, and that these effects may vary in intensity from individual to individual.

Li toxicity depends on the species (model animal) and the time of exposure to it (Shahzad et al., 2017). The serum therapeutic concentration of Li in human ranges from 0.5 to 1 mmol L^{-1}. Signs of mild toxicity are observed in the range from 1.8 to 2.5 mmol L^{-1}, and values higher than 2.5 mmol L^{-1} can lead to severe toxicity (Oruch et al., 2014; Won and Kim, 2017).

The US Environmental Protection Agency (EPA) and some authors recommend a daily dose of approximately 1 mg for an adult of 70 kg (Schrauzer, 2002; Szklarska and Rzymski, 2019). Among these health benefits, Li is known for the ability to stimulate the production of neural stem cells (Zhang et al., 2019), protect against oxidative stress, stimulate the immune system, produce a calming effect, present a neuroprotective effect (Szklarska and Rzymski, 2019), besides its potential to prevent and treat some types of cancer (Ge and Jakobsson, 2019). Studies in different cities have demonstrated that increased Li levels (12 to 160 μg L^{-1}) in drinking water reduce the occurrence of homicide, suicide and neurodegenustive diseases (Kessing et al., 2017; McGrath and Berk., 2017; Barjasteh-Askari et al., 2020). Thus, the present study aimed to evaluate the bioavailability of a Li-enriched mushroom of *Pleurotus djamor* at recommended and therapeutic dosage, besides its effects against oxidative stress, using swine models.

MATERIAL AND METHODS

Production of Li- enriched mushrooms

The *P. djamor* (isolate PLO 13) used in the study belongs to the collection of fungi of the Laboratory of Mycorrhizal Associations/Department of Microbiology/Institute of Biotechnology Applied to Agriculture and Livestock - BIOAGRO/Universidade Federal de Viçosa – UFV. The isolate PLO 13 was grown in Petri dishes containing 20 mL of potato-

dextrose-agar culture medium and kept at 25 ± 1 °C. After seven days, ¼ of the medium growth totally colonized by the fungus was transferred to each 280 mL pot containing 130 g of cooked and autoclaved sorghum grain for 1 h.

The substrate used for the production of the mushrooms was a mixture of coffee husk and sugarcane bagasse (9:1, v/v). The coffee husk was cooked for 2 h and centrifuged at 1500 *g* for 1 min. The sugar cane bagasse was immersed in a 2 % calcium hydroxide solution (w:v) for 12 h and centrifuged at 1500 *g* for 1 min. Next, 1 kg of this mixture was placed in a polypropylene bag and autoclaved for 1 h, at 121 ºC. Then, PLO 13 was inoculated into the substrate with 50 mL lithium chloride, at the concentration of 40 g L^{-1}. After the incubation period, the packages were transferred to a fruiting room with controlled temperature and humidity of 20 ºC and 80%, respectively. After about 25 days, the mushrooms were harvested, dried and grounded.

Lithium content

Nitroperchloric digestion and lithium quantification of the samples was performed according to the methodology of Tedesco et al. (1995).

Assay 1: Pig tolerance to lithium carbonate

The animal assay was carried out in partnership with the pig farm of the Department of Animal Science at UFV. All methods involving the handling of piglets followed the ethical principles of animal research (CONCEA, 2019) and were previously approved by the Commission of Ethics in the Use of Animal Production of the Universidade Federal de Viçosa (Protocol Nº. 02/2021). To evaluate the maximum dosage of Li to be administered, it was used in 24 animals of 28-days-old female piglets (*Sus Domesticus*, AGPIC 415 × Camborough) (Agroceres PIC, Patos de Minas, MG, Brazil), to evaluate their tolerance to lithium carbonate (Li_2CO_3). The triplicate treatments received the following oral doses of lithium carbonate daily, during five days: 0.034; 0.068; 0.136; 0.205; 0.272; 0.350; 0.650 and 0.950 g. The lithium carbonate was weighed and encapsulated in gelatin capsules nº 00. All animals received water and food *ad libidum* and were distributed in a completely randomized design. The animals were observed after oral administration of the capsule to ensure its ingestion. On the sixth day, 10 mL of blood was collected by puncture of the orbital sinus for lithium quantification.

Essay 2: Lithium bioavailability and oxidative stress

Piglets of the same genetics and at the same age (28-33 days-old), as described in the previous item, were used. A total of 28 animals, were distributed in 7 pens, with 4 animals per pen, in a completely randomized design, in a room in which the temperature was maintained within the thermoneutral zone during the experimental period. The piglets had free access to feed and water throughout the five experimental days.

To verify the effect of lithium in its different forms (carbonate or enriched mushroom) and dosages (therapeutic or recommended), the following treatments were performed (Table 1).

Table 1. Treatment and source and amount of lithium provided for each pig

Treatments	Source of lithium	Lithium content (mg/day)
Control	No lithium	0
LiT	300 mg of Li_2CO_3	56*
M+LiT	122 g of Li-enriched mushroom flour	56*
M	122 g of non-enriched mushroom flour	0
LiR	5,7 mg of Li_2CO_3	1**
M+LiR	2,2 g of Li-enriched mushroom flour	1**
LiS	600 mg of Li_2CO_3 (Li overdose)	113

*Recommended and **therapeutic dosage (Schrauzer, 2002; Szklarskaa and Rzymski, 2019)

The dosage (Table 1) were administered dividing into 3 times a day. Treatments M + LiT and M also received empty capsules 3 times a day, so that all animals could be under the same stress conditions. This process was repeated during five experimental days which is the average time that Li takes to stay in balance in the body.

In order to verify how the Li was absorbed by the piglets, 4 mL of blood were collected dail. These samples were submitted to laboratory analysis for Li quantification.

At the end of the experimental period (sixth day), 12 mL of blood were taken from each animal. Then, the animals were electrically stunned, and exsanguination was performed for sample collection of brain, liver and kidney tissues.

Quantification of Li

Brain, liver and kidney tissues were collected and red blood cells were obtained from blood samples taken on the last day of the experiment. The tissue samples were dehydrated at 70 °C until constant weight, ground and homogenized. Soon after, 300 mg of each tissue were

submitted to digestion and quantification of lithium by atomic emission in a flame spectrophotometer (Tedesco et al., 1995). The red blood cells were centrifuged and separated from the serum. Then, 4 mL of 0.9 % NaCl were added to each tube and centrifuged at 3000 rpm, for 15 min. The supernatant was discarded. This washing process was repeated 5 times, and the red blood cell samples were dehydrated at 70 ºC until constant weight and submitted to the same digestion and quantification process of the other tissues.

Urine samples were taken from the bladder of the animals immediately after slaughter and stored at -20 ºC. For the quantification of lithium, 5 mL of urine were diluted in 15 mL of distilled water and subsequently filtered using filter paper. The samples were subjected to quantification by atomic emission in a flame spectrophotometer, using the adapted methodology described by Dol and collaborators (1992). The blood serum samples were subjected to a colorimetric assay and quantified in a flame photometer.

Serum cortisol levels

The serum cortisol was quantified by chemiluminescence test, as described by (Tellez et al., 2006).

Oxidizing activity and oxidative stress markers in tissues

Sample preparation

To prepare the samples, 100 mg of each tissue (brain, liver and kidney) were thawed and homogenized in phosphate buffer 0.2 mol L^{-1}, ethylene diaminetetraacetic acid (EDTA) 1 mmol L^{-1}, pH 7.4, using a homogenizer. The homogenates were centrifuged at 15,000 *g* for 10 min, at 4 °C, and the supernatants were used for analysis of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and malondialdehyde (MDA).

Superoxide dismutase activity (SOD)

The SOD activity was determined by the method based on the reduction of superoxide (O^{-2}) and hydrogen peroxide (Dieterich et al., 2000).

Catalase activity

Catalase activity (CAT) was determined according to Aebi, (1984), using hydrogen peroxide (20 mmol L^{-1}) as substrate.

Glutathione S-transferase activity (GST)

GST activity was measured using the method of Habig and collaborators (1974).

Determination of malondialdehyde

The extent of lipid peroxidation (OLP) was measured using malondialdehyde (MDA), which is the main product of lipid peroxidation (Buege and Aust, 1978).

Statistical analysis

The data distribution was determined by the Shapiro-Wilk test, using the Minitab program (version 9.0). The data were submitted to analysis of variance (ANOVA), followed by the Fisher test for multiple comparisons. Statistical significance was established at $p < 0.05$. When relevant, the data were submitted to regression analysis by the R program.

RESULTS

Mushroom enrichment

Mushrooms have proved to be great sources of minerals. *Pleurotus djamor* PLO 13 was managed to accumulate 464 μg of Li per gram of dry mushroom (de Souza Lopes et al. 2022). So, this fungus presented the greatest bioaccumulation among the fungi already studied, which makes it an alternative to supply higher doses of Li, mainly for human therapeutic use.

Essay 1: Pig tolerance to lithium carbonate concentration

This is the first information regarding the tolerance of piglets to lithium salt. Serum Li concentration in piglets did not differ in the first 3 Li_2CO_3 dosages (0.034, 0.068, 0.136 g) ($p>0.05$), and did not exceed 0.25 mmol L^{-1} (Figure 1). Increased lithemia was observed for the dosage above 0.205 g of Li_2CO_3. At the dosage of 0.650 g Li_2CO_3, the animals presented mild signs of intoxication, such as fine tremors and diarrhea, when the serum concentration of Li was 2.90 mmol L^{-1}.

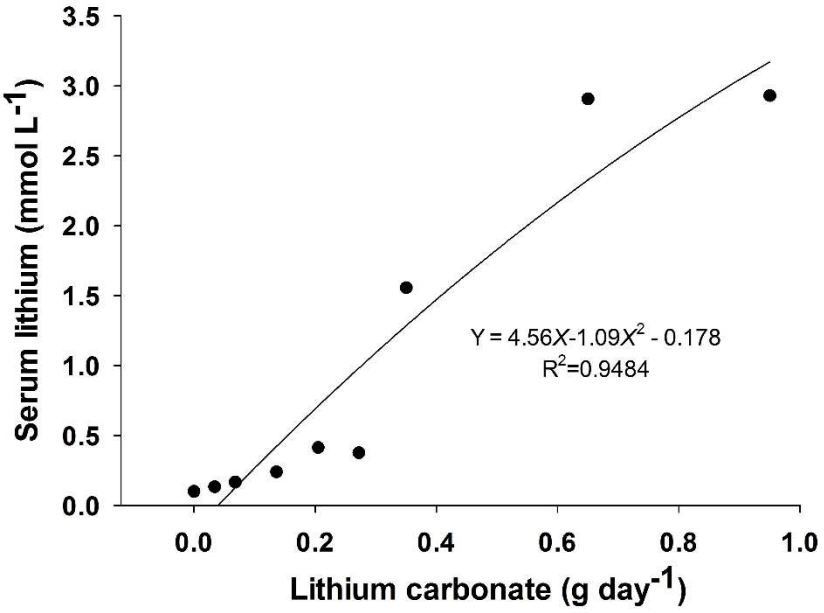

Figure 1. Serum lithium concentration in piglets fed with different doses of Li_2CO_3 after five days.

Essay 2: *In vivo* lithium bioavailability and oxidative stress

The Li present in the blood serum of the piglets could be quantified only in the animals that received this mineral in the enriched mushroom (M+LiT) at the corresponding dosage of 56 mg of Li per day and in the animals that received 300 mg of Li_2CO_3 (LiT -56 mg of Li) and 600 mg of Li_2CO_3 (LiS - 113 mg of Li) per day. For the other treatments, the serum of the piglets presented no Li or Li concentrations was below the limit of detection of the technique (0.10 mmol L^{-1}). As seen in Figure 2, the three treatments showed an increase in serum Li over the days, and equilibrium was reached after the third day. The highest concentrations of serum Li were found in animals that received the highest dosage of Li (600 mg) in the form of Li_2CO_3. When comparing the therapeutic doses (M+LiT and LiT) we can see that the highest levels of serum Li were from the enriched mushroom (M+LiT), that is, the bioavailability of Li is higher in this treatment.

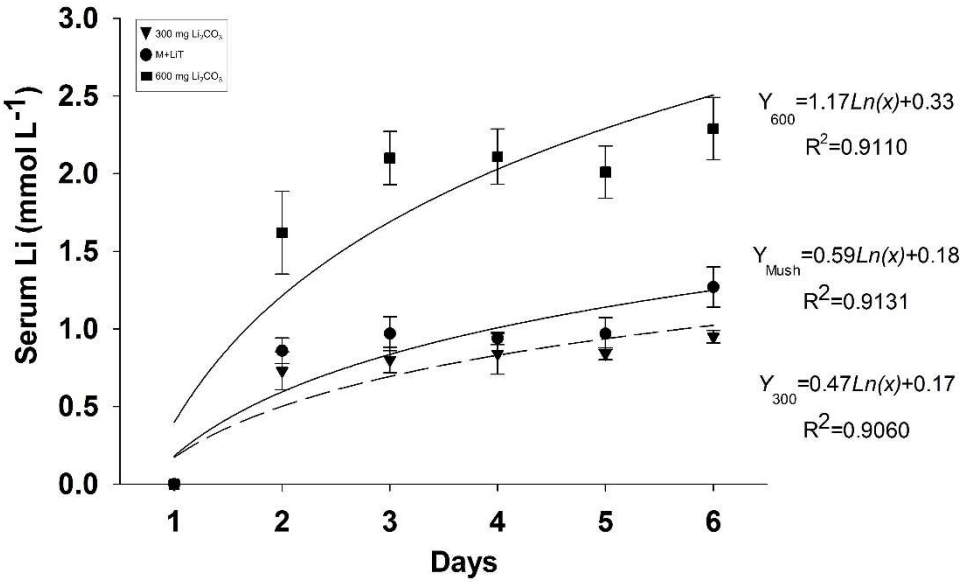

Figure 2. Kinetics of lithium absorption in the blood of piglets fed for 6 days. ■ animals that received 600 mg of Li_2CO_3 per day (113 mg of Li); ● animals that received 122 g of Li-enriched mushroom (56 mg of Li). ▼ animals that received 300 mg of Li_2CO per day (56 mg of Li).

Li in tissues

Li was not found or was presented in levels below the detection limit of the device in the tissues of the control animals and treated with non-enriched mushroom (M). This result was expected, since Li was not added either to the meal or the growth substrate of the mushroom.

In all tissues and serum (Figure 2 and 3), Li was detected in the animals treated with Li-enriched mushroom (M+LiT) and Li_2CO_3 (LiT), both corresponding to a daily dose of 56 mg of Li. Serum concentrations of this element were around 1.3, 1.0 and 2.3 mmol L^{-1} for the treatments M+LiT, LiT and LiS, respectively (Figure 2). In the urine, in the same treatments, the values were approximately 1.5 for the first two and 2.4 mmol L^{-1} for the last one (Figure 3e). There was no difference between these two treatments, which reveals that Li obtained from mushroom presents the same accumulation in the tissues as LiT. The highest concentration of Li (1.22 mmol L^{-1}) was found in blood serum when piglets were fed with M+LiT. Among the tissues, the kidney accumulated the highest concentration, about 6 ng of Li per gram of dry tissue. For animals fed with LiS, the serum presented the highest concentration of Li, followed by the kidney, liver, brain and erythrocytes (Figure 3).

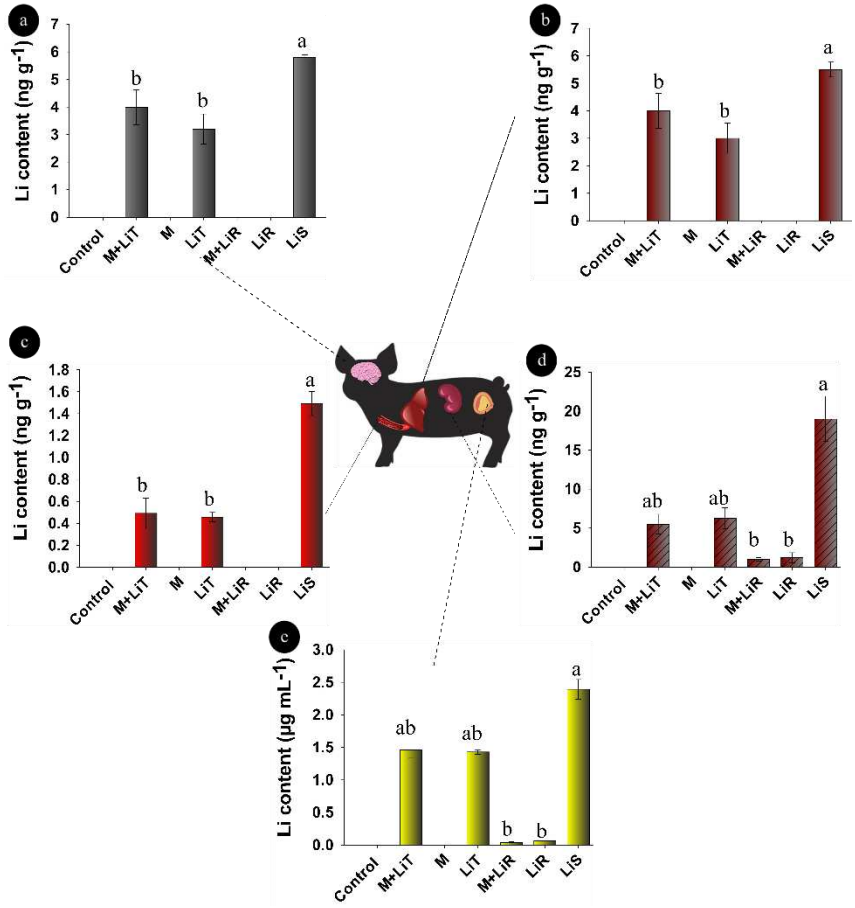

Figure 3. Quantification of Li in the brain (a), liver (b), erythrocytes (c), kidney (d) and urine (e). Means that do not share the same letter are statistically different by Fisher's test ($p < 0.05$).

Serum cortisol levels

Piglets that received LiS, which were administered at dosages of 650 mg Li_2CO_3 and reached a serum concentration of 2.3 mmol L^{-1} of Li, presented the highest serum cortisol values (Figure 4). The animals presented clear signs of Li poisoning, such as tremors and diarrhea. The second highest dose of cortisol was observed in piglets that did not receive any Li or mushroom. The lowest dosages were identified for the animals that received LiT or M+LiR, which received, respectively, 56 mg of Li and 2.2 g of enriched mushroom (1 mg of Li), while other treatments presented no differences ($p>0.05$) in serum cortisol levels (Figure 4).

At lower dosages, as in the case of LiT and M+LiT, Li seems to have a negative effect on cortisol production. It is noteworthy that, when provided in the form of mushroom flour in the recommended daily dosage (1mg of Li per day), even far below the dosage of the LiT treatment (56 mg of Li per day), tranquilizing effects are achieved.

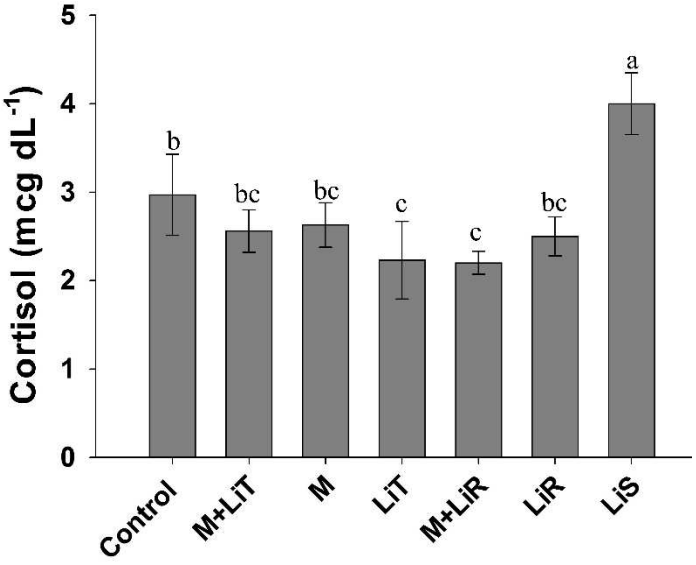

Figure 4. Levels of serum cortisol in pigs. Means that do not share a letter are statistically different by Fisher's test ($p < 0.05$). Control: animals treated only with meal; M+LiT: animals treated with enriched mushroom (56 mg of Li); M: animals treated with non-lithium-enriched mushroom; LiT: animals treated with 56 mg of Li (300 mg of Li_2CO_3); M+LiR: animals treated with enriched mushroom (1 mg of Li); LiR animals treated with 1 mg of Li (5.7 mg of Li_2CO_3); LiS: animals treated with 113 mg of Li (600 mg of Li_2CO_3).

Enzymatic antioxidants and oxidative damage markers

Brain

The highest levels of SOD in the brain were found in treatments that received Li, regardless of the dosage or form provided (Li_2CO_3 or enriched mushroom) (Figure 5a). Animals that did not receive Li presented lower levels of this enzyme, which demonstrates that this mineral can stimulate SOD activity. In the other hand, lower levels of CAT were found in the treatments with enriched mushroom in the therapeutic and recommended dosage (M+LiT and M+LiR) (Figure 5b). The treatments with enriched mushroom seem to have a lower effect on CAT when compared to the other forms of Li provided, mainly at higher dosage, as is the case of the LiS treatment (Figure 5b).

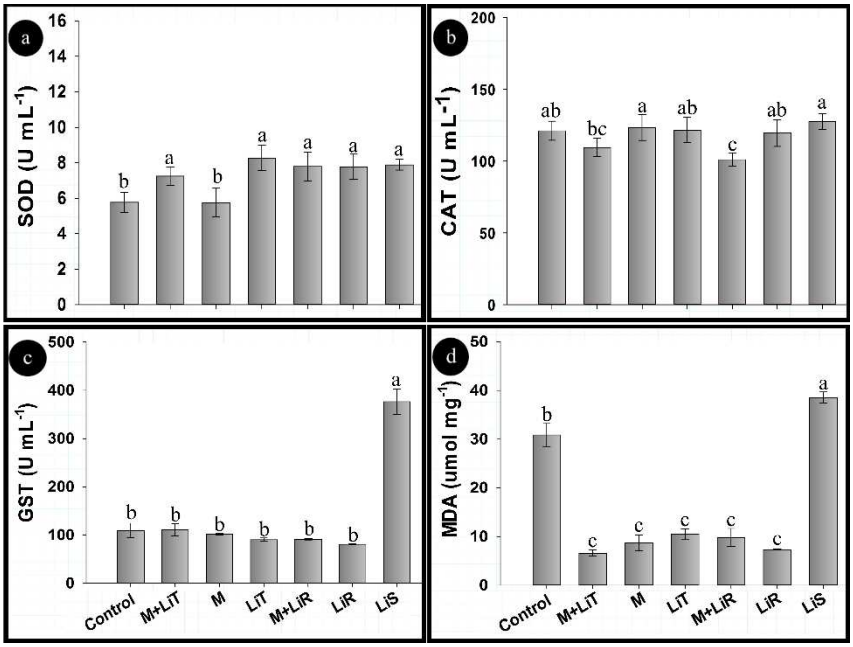

Figure 5. Enzymatic antioxidants and oxidative damage markers in the brain of piglets administered with different sources of Li. (a) Superoxide dismutase activity; (b) Catalase activity; (c) Glutathione S-transferase activity and (d) levels of malondialdehyde. Means that do not share the same letter are statistically different by Fisher's test ($p < 0.05$).

For GST, there was difference between the LiS treatment and the others, which reveals an increase of about 4 times in its activity (Figure 5c).

MDA levels are higher in Control animals and LiS (Figure 5d), which reveals that these groups suffered the most oxidative damage and that, at therapeutic dosage (M+LiT and LiT) and recommended daily dosage (M+LiR and LiR).

Liver

The liver enzymes SOD and CAT presented similar behavior between treatments (Figure 6a and 6b). SOD presented the highest activity in the LiS treatment and than M+LiT, although the later did not differ from M and LiT ($p>0.05$; Figure 6a). CAT also presented the highest levels for LiS, than for M+LiT, in which the later differed only from the control ($p<0.05$; Figure 6b). Liver GST didn't change according to the Li administration ($p>0.05$; figure 6c).

The treatments that exhibited greater damage related to lipid peroxidation, and consequently higher values for MDA are in the Control, M and LiS (Figure 6d). The lowest values of MDA were quantified in M+LiT and LiT, which reveals that, regardless of the form (Li-enriched mushroom or Li_2CO_3), this dosage of Li can prevent damage to the liver than other treatments.

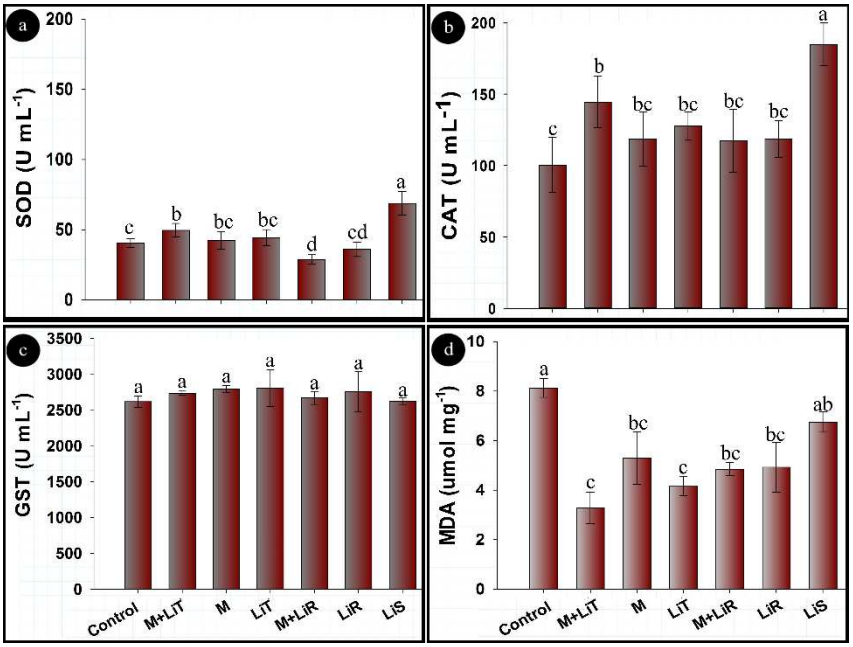

Figure 6. Enzymatic antioxidants and oxidative damage markers in the liver of piglets administered with Li. (a) Superoxide dismutase activity; (b) Catalase activity; (c) Glutathione S-transferase activity and (d) levels of malondialdehyde. Means that do not share the same letter are statistically different by Fisher's test ($p < 0.05$).

Kidney

The lowest value of renal SOD activity was obtained for the control, but this differed only from the LiR treatment ($p<0.05$; Figure 7a). CAT higher activity were observed for treatments M+LiT and M, which did not differ ($p<0.05$) from LiR and LiS (Figure 7b). For GST, the greatest activity was found for the M+LiT treatment, which differed from all other treatments, including LiT, which received the same dosage of Li but in the form of Li_2CO_3 (Figure 7c).

The lowest value for MDA was also found for the M+LiT treatment. The control and LiT were the treatments that suffered the greatest oxidative damage (Figure 7d).

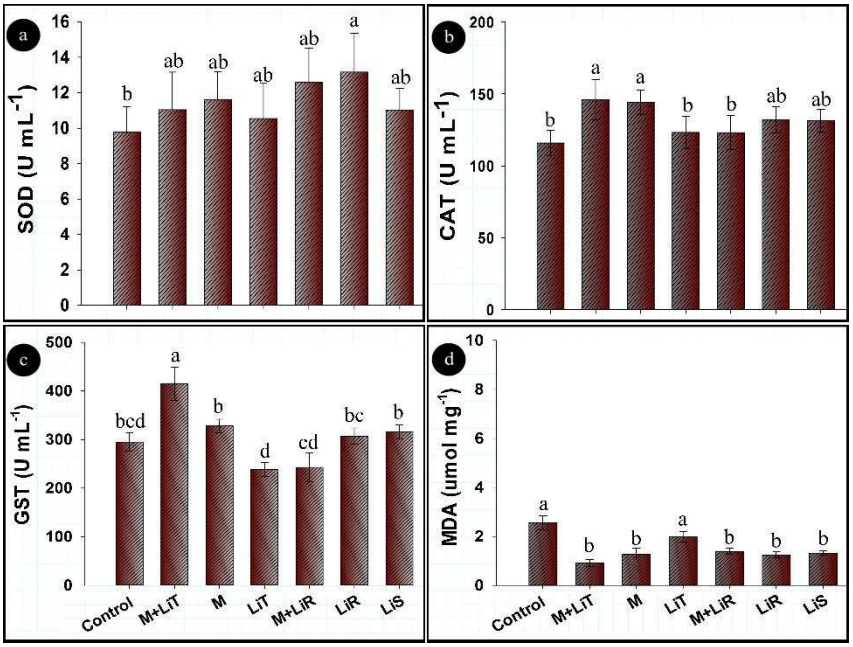

Figure 7. Enzymatic antioxidants and oxidative damage markers in the kidney of piglets administered with different sources of Li. (a) Superoxide dismutase activity; (b) Catalase activity; (c) Glutathione S-transferase activity and (d) levels of malondialdehyde. Means that do not share the same letter are statistically different by Fisher's test ($p < 0.05$).

Serum

The quantifications of antioxidant enzymes in blood serum did not differ between treatments ($p > 0.05$), except for GST, in which the lowest and highest values of their activity were those of the Control and LiS, respectively, which differentiated them from the other treatments (Figure 8c). Apparently, the administration of Li at dosages from the therapeutic level, and unenriched mushrooms, stimulates the activity of these enzymes.

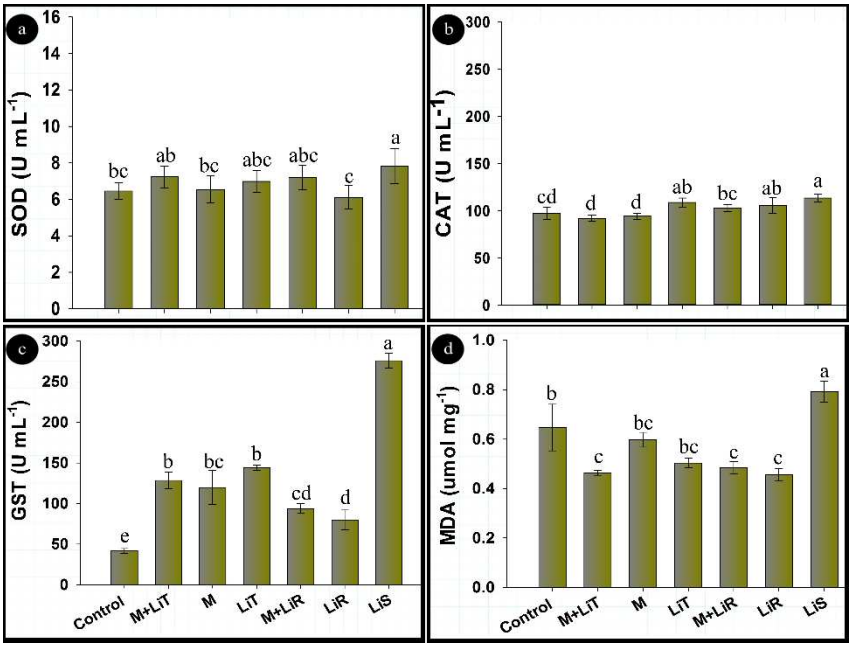

Figure 8. Enzymatic antioxidants and oxidative damage markers in the blood serum of piglets administered with different sources of Li. (a) Superoxide dismutase activity; (b) Catalase activity; (c) Glutathione S-transferase activity and (d) levels of malondialdehyde. Means that do not share the same letter are statistically different by Fisher's test ($p < 0.05$).

The analysis of the stress markers demonstrates that the lowest levels of MDA were genusted in the M+LiT, LiT and M+LiR treatments, but they did not differ from the other treatments that received some form of Li and mushroom and the control, except for the LiS that presented the highest levels of MDA (Figure 7d).

DISCUSSION

Pleurotus djamor confirmed is a very good accumulator of Li and tolerates high doses of this component. There are works showing that *Pleurotus ostreatus* grown in coffee husk bioaccumulated about 200 µg (de Assunção et al., 2012) and 37.5 µg (Vieira et al., 2013) of lithium per gram of dry mushroom. The solubility and availability of lithium present *in P. djamor* PLO 13 can be controlled through its association with organic compounds as observed for other ions, which can be digested and released slowly during digestion, which reduces the risk of intoxication and/or other collaterals effects (Scheid et al., 2020).

There is no work, in consulted literature, demonstrating the tolerance of piglets (Figure 1) to any lithium salt. The same symptoms of intoxication found in our work have been observed in humans, which has observed that in who make daily use of Li_2CO_3 the serum Li concentration ranges from 1.8 to 2.5 mmol L^{-1} (Oruch et al., 2014; Won and Kim, 2017). At the

higher dosage of 0.950 g of Li_2CO_3 per day, the Li concentration in the serum reached 2.91 mmol L^{-1} (Figure 1), where the animals presented severe signs of Li intoxication, such as muscle weakness, shaky limbs, loss of appetite and diarrhea. The intoxication symptoms are also observed in humans with litemia above 2.5 mmol L^{-1} (Oruch et al., 2014; Won and Kim, 2017). Lithium toxicity can induce lipid peroxidation in neurons, which leads to serious disorders (Efendiev and Kerimov, 1994; Yao et al.,1999; Yousefsani et al., 2020). A recent study demonstrated that Li alters the membrane potential in the mitochondria and increases the production of reactive oxygen species, which damages various cellular structures (Yousefsani et al., 2020).

The behavior of lithium in the porcine organism is similar to that of humans, and its toxicity depends on the species affected and the time of exposure to Li (Shahzad et al., 2017). Although the serum Li values for toxicity are close to those of humans, we must take into account the dosage used and the weight of the animals. The animals used in the experiment have a mass of approximately 7 kg. An adult of 70 kg who makes therapeutic use of some lithium salt consumes 600 to 1200 mg of Li_2CO_3 daily Li (Shahzad et al., 2017). Taking into account the 7 kg weight of the animals, the therapeutic dosage, when compared to that of a human, would be 10 times lower (about 60 to 120 mg of Li_2CO_3). However, we can observe that 10 times higher doses were necessary to reach serum therapeutic and overdose levels similar to those of humans, which may indicate that these animals are more tolerant to high doses of Li when compared to humans.

So far, this is the first *in vivo* experiment that demonstrates that Li-enriched mushrooms are a source more bioavailable than Li_2CO_3 and that, in addition to being considered a healthy food, it can be used as a source of Li. Besides, it can be a functional food and can provide pharmacological benefits. Bioavailability is the proportion of a nutrient in a food that is available to the body and reaches the bloodstreamand it can be used in bodily functions (Uribe et al., 2020). To date, no study has demonstrated the bioavailability of Li of Li-mushrooms in the body. There are *in vitro* studies demonstrating that the Li present in the enriched mushroom is more bioaccessible to the organism when compared to Li_2CO_3 (de Assunção et al., 2012). In the *in vitro* study by Scheid et al. (2020), the mycelium of different species of fungi enriched with Li presented increased bioaccessibility in relation to the Control (unenriched mushrooms). A similar result was observed, *in vivo*, for Se- enriched mushroom of *P. ostreatus* (da Silva et al., 2010; Bhatia et al., 2013).

Lithium is almost completely absorbed in the small intestine when administered in the form of its salts. After absorption, it is evenly distributed throughout the body fluid (Wen et al., 2019). Our results show that in piglets the Lithium was accumulated in higher concentration in kidneys then in other investigated tissues (Figure 3), similar to observed in rats administered with LiCl, where kidney was among the tissues that accumulated most Li (Garcíaa et al., 1999). The differences in Li concentration among the tissues can be explained by the characteristics of the plasma membrane of the cells in each tissue, which can affect the transport of that ion (Garcíaa et al., 1999). Li positively helps in the oxidative defense of the organism. However, dosages above the therapeutic level or its non-administration (Control) have a negative effect.

It is worth mentioning that only the renal tissue presented quantification of Li in the M+LiR and LiR treatments with a dosage of 1 mg of Li per day (figure 3), the recomended dosage (Schrauzer, 2002; Szklarska and Rzymski, 2019).

Our data corroborated the findings of other authors (Iwai et al., 2021), who observed that Li concentrations in the urine reflected the serum ion concentration in human. Human and piglets' serum and urine presented a positive correlation between serum Li and the Li excreted in the urine. After being administered orally, Li is absorbed and excreted in the urine and has a half-life of 12h (Wen et al., 2019).

Cortisol is a hormone synthesized by the adrenal glands and is one of the most used biomarkers to assess stress in piglets (Martínez-Miró et al., 2016). At high doses, Li stimulates the production of cortisol and at lower doses (and control) Li seems to have a negative effect on the production of the same hormone (Figure 4). A study with humans detected that increased cortisol production was related to increased Li dosage in individuals with bipolar disorder (Bschor et al., 2020). Some studies have revealed that the presence of Li in drinking water (at levels ranging from 12 to 160 μg per liter of water) reduces homicide, suicide and related diseases (Knudsen et al., 2017; Barjasteh-Askari et al., 2020). Similarly, in a study with human affected with bipolar disorder, who were using Li therapy presented lower serum cortisol levels than those did not use this drug (Mühlbauer and Müller-Oerlinghausen, 1985).

The antioxidant defenses presented their activit influenced by the dose, source and tissue analyzed. Among the analyzed tissues, the liver showed the highest levels of the analyzed antioxidant enzymes (Figure 6a and b). In a study with rats, it was found that the animals that were administered with Li presented higher activity of the enzymes SOD and CAT. However, this increase can be related to a higher level of oxidative stress due to the high levels of MDA

(Nciri et al., 2009; Nciri et al., 2012). In our study, although the activity of these enzymes is higher in treatments with Li, as in the case of M+LiT, in this treatment lipid peroxidation was lower, compared to Control and LiS (high dosage), which indicates that this dosage and form of Li prevent oxidative damage to the liver when compared to Control and LiS (Figure 6d).

The GST had a different behavior depending on the tissue analyzed in which the brain was the one that showed difference among the treatments in which LiS presented levels 4 times higher when compared to the other treatments. This may indicate an oxidative damage in the tissues of these animals (LiS) which is evidenced by the high levels of MDA in them. In a study with rats, higher levels of cerebral GST were found at higher dosages of Li (Shao et al., 2008), but no dosage exceeded the therapeutic dosage, as is the case with LiS treatment of our study. Analyzing neural cells of rats, it was found that the genustion of reactive oxygen species increased with increase of dosage of Li_2CO_3 (Yousefsani et al., 2020). In genusl, the highest MDA levels were found in the LiS treatment in all tissues, except for the kidneys, which had the highest MDA levels in the Control that received neither Li nor mushroom, maybe lithium or mushroom is protecting kidneys against lipid peroxidation.

Comparing the results obtained from the M+LiT treatment for antioxidant enzymes (which in genusl had the greatest activities) with the levels of MDA, we conclude that the enriched mushroom, in addition to increasing the activity of these enzymes, is also a protective agent against oxidative stress in analyzed tissues.

CONCLUSIONS

This is the first *in vivo* report on the bioavailability of Li-enriched mushrooms in piglets. The findings demonstrate that the Li-enriched mushroom is a more bioavailable source of Li when compared to lithium carbonate. Cortisol is an important marker of stress responding well to Li concentrations. The activity of enzymes is influenced by the concentrations and forms of Li provided and MDA proved to be a coherent marker of oxidative stress in piglets. Thus the Li-enriched mushrooms of the isolate of *P. djamor,* PLO 13, present high potential and can be an alternative source for suppling this mineral in the diet of humans, as a food supplement, due to its various beneficial properties to the organism or even in the therapeutic treatment of diseases that use to use Lithium.ug.

Acknowledgments

The authors are grateful to the Cordenação de Aperfeiçoamento de Pessoal de Nível superior (Capes-001), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) for their financial support.

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CAPÍTULO 3

Intestinal microbial diversity of swines fed with different sources of lithium

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ABSTRACT

The gut is an ecosystem with one of the largest known microbial populations. Microorganisms can be harmful, but many of them are related to the health of the host, as they can release metabolites that can fight pathogens, stimulate the immune system and even synthesize signaling molecules that reach the central nervous system triggering responses. A drug that is widely used in the treatment of psychiatric disorder is lithium in its salt forms, in which lithium carbonate is the most used. Despite being widely used, people who make the therapeutic use of some lithium salt develop a series of side effects. Through metataxonomic data we sought to understand whether lithium in its carbonate form and in Li-enriched mushrooms could influence the microbial composition of the ileum, colon and feces of piglets. By means of the bray curtis metric, no difference was observed between the evaluated treatments, while the alpha diversity indices showed difference in the Simpson, Shannon and Chao-1 indices in the colon and Chao-1 in the feces, depending on the treatment, particularly those treatments that received lithium, when compared to treatments that received no form of lithium. The taxonomic grouping of amplicon sequence variants (ASVs) showed that the taxa with the highest relative abundance can vary between the ileum, colon and feces, with a predominance of phyla such as *Firmicutes*, *Bacteroidota* and *Proteobacteria* in treatments that receive lithium. Many groups of microorganisms that are important for the health of the host have had their relative abundance enriched. *Lactobacillus, Ruminococcaceae*, *Enterorhabdus*, *Muribaculaceae* and *Coprococcus* had their relative abundance increased in animals that received the recommended dose of lithium (M+LiR). At the recommended dosages, there was an increase in the abundance of *Prevotellaceae* and *Bacteroidales* (treatments that received enriched mushroom) and *Clostridia*, *Ruminococcus, Burkholderia* and *Bacteoidales* (treatments that received lithium carbonate). In all the groups mentioned above, their low abundance is associated with people with some mood disorder. This is the first work to show the effects of lithium and lithium-enriched mushrooms on the composition of the intestinal microbiota in piglets and that this change in the composition of the microbiota may have implications for the health of the host, suggesting that one of the pathways of lithium's effects may be related to this ability to alter the intestinal microbiota.

Keywords: Disorder, piglets, metataxonomy, dysbiosis, organic acids

INTRODUCTION

The human gastrointestinal tract harbors a microbial community estimated at 100 trillion microorganisms composed of the most diverse groups of bacteria, archaea, fungi and viruses (Thursby and Juge, 2017; Rinninella et al. 2019) with more than 3 million genes (about 130 times more than the human genome) and producing thousands of metabolites (Valdes et al., 2018). Microbial phyla of the digestive tract were found with a predominance of *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteri*a and *Verrucomicrobia*, the first two representing 90% of the microbiota present in the intestine (Vasilescu et al. 2022). The same authors also points out that *Firmicutes* are represented by genus such as *Clostridium*, *Lactobacillus*, *Bacillus*, *Enterococcus* and *Ruminicoccus*, in which the first represents 95% of this phylum in the human gastrointestinal tract.

The gut microbiota produces thousands of metabolites that can influence the host (Valdes et al., 2018). In addition, it can modulate immune responses, digest food, production of intestinal hormones, neurological signaling, modifying the action of drugs and toxins in the host (Fan and Pedersen, 2021). Through bidirectional communication, the nervous system communicates with the enteric system and its microorganisms (brain-intestinal microbiota axis) that can produce neuroactive compounds such as neurotransmitters, hormones, amino acids, short-chain fatty acids that can influence the host's metabolism (Morais et al., 2021). The same author also points out that the intestinal microbiota can also influence the integrity of the intestinal barrier that controls the passage of signaling molecules from the intestinal lumen to the blood circulation, The integrity of the intestinal barrier can be interrupted in some neuropsychiatric diseases that are related to the abundance of some groups of microorganisms present in the intestine. Many studies have shown that the composition of the gut microbiota in individuals with various neurological diseases is different compared to healthy individuals (Jiang et al., 2015; Morais et al., 2021).

Factors such as age, sex, type of delivery, exposure to stress and diet can cause changes in the composition of the gut microbiota. In the latter case, the gut microbiota can vary greatly between individuals who have a high-protein diet (meat) and individuals who eat a diet rich in carbohydrates and fiber (vegetables, legumes, mushrooms) (Rinninella et al.2019). Some studies have shown that the gut microbiota can be affected by some neuropsychiatric disorders (anxiety and depression) and consequently affect gastrointestinal functions (Huang et al. 2022; Koloski et al. 2012; Gracie, Guthrie, Hamlin, and Ford, 2018). Likewise, some drugs used to treat these disorders can positively modify the gut microbiota, protecting the intestinal mucosa and regulating immune cells (Maier et al. 2018; Song et al. 2020; Huang et al. 2022).

One drug that is widely used in the treatment of disorders such as uni and bipolar disorder and in the prevention of suicide are lithium salts, mainly in their lithium carbonate (Li_2CO_3) form (Cammarota et al. 2020). The same authors emphasize that the administration of these salts, mainly in the form of carbonate, can genuste side effects such as nausea, diarrhea, excessive urination and thirst, hand tremor, weight gain, cognitive impairment, sexual dysfunction, dermatological problems, kidney dysfunctions. Regarding its toxicity, it will depend on the species affected and the time of exposure to Li (Shahzad et al., 2017).

Edible mushrooms, in addition to providing several health benefits, are also considered probiotics, as they induce the growth or action of microorganisms that contribute to the well-being of their host (Jayachandran, Xiao and Xu, 2017). There are works showing that mushrooms when grown in a substrate with the presence of selenium, and/or other minerals, convert selenium into a more bioavailable and less toxic form for the organism (da Silva et al.,2010; Kora,2020). There are works showing that lithium is more bioavailable and bioaccessible when the source is the mushroom enriched with this mineral (de Assunção et al., 2012; de Souza Lopes et al.,2022). This demonstrates the biotechnological potential of Li-enriched mushrooms as an alternative source of this mineral and can even minimize the toxic effects of people who make the therapeutic use of this element.

The United States Environmental Protection Agency (EPA) and some authors recommend a daily dose of approximately 1 mg for a 70 kg adult (Szklarska and Rzymski, 2019). Among these health benefits, lithium (Li) is known for its ability to stimulate the production of neural stem cells (Zhang et al. 2019), protect against oxidative stress, stimulate the immune system, produce a calming effect, have a neuroprotective effect (Szklarska and Rzymski, 2019), in addition to its potential to prevent and treat some types of cancer (Ge and Jakobsson, 2019). The therapeutic serum concentration of Li in humans is in the range of 0.5 to 1 mmol L^{-1}. Signs of mild toxicity are seen in the range of 1.8 to 2.5 mmol L^{-1}, and values greater than 2.5 mmol L^{-1} can lead to severe toxicity (Won and Kim 2017).

Lithium has an effect on and/or can change the diversity and composition of the gut microbiota (Lieb, 2004; Cussotto et al., 2019; Huang, 2022). However, these authors did not analyze the microbiota subjected to different sources and dosages of lithium. So, the objective of this work is to evaluate the diversity and composition of the intestinal microbiota in the ileum, colon and feces of piglets submitted to different dosages (recommended, therapeutic and overdose) and sources of Li (no Li, Li-enriched mushroom and Li_2CO_3).

MATERIAL AND METHODS

Inoculum and enriched mushrooms

The *Pleurotus djamor* (strain PLO 13) used in the study belongs to the collection of fungi of the Laboratório de Associações Micorrízicas, Departamento de Microbiologia, Instituto de Biotecnologia Aplicada à Agropecuária - BIOAGRO/Universidade Federal de Viçosa – UFV. The isolate PLO 13 was grown in Petri dishes containing 20 mL of potato-dextrose-agar culture medium and kept at 25 ± 1 °C at 25 °C. After seven days, ¼ of the plate totally colonized by the fungus was transferred to each 280 mL pot containing 130 g of cooked and autoclaved sorghum grain.

The substrate used for the production of the mushrooms was a mixture of coffee husk and sugarcane bagasse (9:1, v/v). The coffee husk was boiled in water for 2 h and centrifuged at 1500 g for 1 min. The sugar cane bagasse was immersed in a 2 % calcium hydroxide solution (w:v) for 12 h and centrifuged at 1500 g for 1 min. Next, 1 kg of this mixture was placed in a polypropylene bag and autoclaved for 1 h, at 121 ºC. Then, PLO 13 was inoculated into the substrate with 50 mL lithium chloride, at the concentration of 40 g L^{-1}. After about 25 days, the mushrooms were harvested, dehydrated to constant weight and grounded. The quantification of Li in mushrooms was done by atomic emission (Tedesco et al. 1995).

Animal assay

The animal test was carried out in partnership with the Department of Animal Science at UFV in the swine sector. All methods involving the handling of piglets followed the ethical principles of animal research (CONCEA, 2019) and were previously approved by the Commission of Ethics in the Use of Animal Production of the Universidade Federal de Viçosa (Protocol No. 02/2021). We chose piglets as an animal model for this work because of their metabolic and physiological similarity with humans (Yang et al., 2016), since lithium is used in the treatment of psychiatric diseases and the results of this work can be extrapolated to humans.

Twenty-four 28-days female piglets were used (Sus Domesticus, AGPIC 415 × Camborough) (Agroceres PIC, Patos de Minas, MG, Brazil). The animals were distributed in 7 pens, with 4 animals per pen, in a completely randomized design, in a room in which the temperature was maintained within the thermoneutral zone during the experimental period. The piglets had free access to feed and water throughout the five experimental period (28–33 days of age).

To verify the effect of lithium in its different forms (carbonate or enriched mushroom) and dosages (therapeutic or recommended), the following treatments were performed (Table 1).

Table 1. Treatment and source and amount of lithium provided for each pig

Treatments	**Source of lithium**	**Lithium content (mg/day)**
Control	No lithium	0
LiT	300 mg of Li_2CO_3	56*
M+LiT	122 g of Li-enriched mushroom flour	56
M	122 g of non-enriched mushroom flour	0
LiR	5,7 mg of Li_2CO_3	1**
M+LiR	2,2 g of Li-enriched mushroom flour	1
LiS	600 mg of Li_2CO_3 (Li overdose)	113

*Recommended and **therapeutic dosage (Szklarskaa & Rzymski, 2019)

All treatments were administered 3 times a day, that is, the values mentioned (Table 1) were divided into 3 dosages. Treatments M + LiT and M also received empty capsules 3 times a day, so that all animals could be under the same stress conditions. This process was repeated during five experimental days. Then, the animals were electrically stunned and exsanguination was performed to collect samples of the ileum, colon and feces.

Extraction, sequencing and analysis of sequences

Intestinal (colon and ileum) and fecal samples were collected shortly after slaughter, which was performed by electronarcosis stunning and brachiocephalic trunk bleeding. Total DNA extraction from the samples was performed according to the methodology proposed by Stevenson and Weimer (2007). The quality and quantity of extracted DNA were measured using nano DropTM Plate, and stored at -20 °C until use.

DNA was sequenced using the Illumina method. The sequences were demultiplexed and trimmed to remove primers, barcodes, and adapters. All reads with a maximum expected error of one or more were removed to keep only high-quality sequences. Then, we removed all chimeras and singletons. The remaining sequences were clustered in Amplicon Sequence Variants (ASVs) (Callahan et al., 2016). Each ASV was annotated using a pre-trained algorithm (classify-sklearn) with the SILVA V.138 database. All sequences annotated as organelles (mitochondria, chloroplasts) were removed from the upstream analyses. All analyses were performed using Qiime2 version 2020.8 (Bolyen et al., 2019).

Concentration of organic acids

The quantification of organic acids was performed with fecal samples from piglets. For analysis, stool samples (~200 mg) were homogenized in 800 µL of Milli-Q water with the aid of vortex and centrifuged at 12,000g for 10 min. The supernatant was removed and the other steps were performed as described by Siegfried, Ruckemann, and Stumpf (1984). The samples were analyzed by high performance liquid chromatography (HPLC), using a Dionex Ultimate 3000 Dual chromatograph coupled to a Shodex RI-101 refractive index (IR) detector maintained at 40 ˚C, and Phenomenex Rezex ROA ion exclusion column, 300 × 7.8 mm maintained at 40 ˚C. The mobile phase used was sulfuric acid (H_2SO_4) 5 mM with flow of 0.7 mL min^{-1}. Acetic, propionic, butyric, and lactic acids were used as standards in the calibration curve. The measurements were performed in duplicate.

Statistical analysis

Differences between groups were evaluated by the ANOVA or Kruskal-Wallis test, followed by Bonferroni's post hoc test ($p \leq 0.05$). The figures were genusted in the GraphPad Prism program (GraphPad Software, San Diego California USA) (Prism, 1994). Normality was tested by the Shapiro-Wilk test for all variables analyzed. Alpha diversity indices were calculated using PAST software (Hammer et al., 2001), and group differences were analyzed in Minitab v.5, using the Kruskal-Wallis test. Beta diversity analyses to compare the microbial composition were assessed at the level of ASVs. The Non-metric multidimensional scale (nMDS) was used to beta diversity analyses based on the Bray-Curtis paired distance and nonparametric similarity analysis (ANOSIM) with permutation number 10,000 and the aid of the PAST software (Hammer et al., 2001). Differences in the relative abundance of ASVs was evaluated by the Kruskal-Wallis test, using the software STAMP v 2.1.3 (Statistical Analysis of Taxonomic and Functional Profiles Statistical Analysis of Taxonomic and Functional Profiles) (Parks et al., 2014). For analyses of relative abundance at phylum, family and genus level, the Wilcoxon test was used to detect intra-group differences after the intervention, differences between groups were analyzed by the Kruskal- Wallis test. The p-values were adjusted using Benjamini-Hochberg's False Discovery Rate (FDR). Values of $P < 0.05$ and $P\_FDR < 0.05$ were considered significant in all analyzes. The analyses were performed in the Minitab program (version 5).

Intergroup gender-level differences in each treatment were analyzed by the linear discriminant analysis (LDA) effect size method (LEfSe) (Tomas et al.,2011) with default settings at https://huttenhower.sph.harvard.edu/galaxy/root.

RESULTS AND DISCUSSION

Sequencing

A total of 10.639.088 crude sequences were genusted with an average length of 467 bp in all samples. After cutting, quality filtering and chimera removal, 9.878.563 high quality bacterial sequences were obtained. The Goods coverage in the samples was >97% indicating that our sequencing efforts sufficiently covered the diversity of bacterial communities in the ileum, colon and feces of piglets. The summary of sequence counts and ASVs that passed through the filtering, cleaning, and normalizing steps are shown in Supplemental Table S1.

Influence of lithium on the composition of the microbial community

This is the first study to analyze the composition of the microbial community of piglets treated with different doses and sources of lithium in the form of lithium carbonate and mushroom enriched with the same element.

By the taxonomic analysis of bacterial communities from the ileum, colon and feces compartments of piglets were observed 19.339 ASVs that were assigned to 36 phyla, 88 classes, 175 orders, 273 families, 562 genus.

Beta diversity analysis showed that the Bray-Curtis differences of bacterial communities were not grouped by treatments (ANOSIM, $p > 0.05$) (Figure 1). This is a result after 5 days and this time may not have genusted a noticeable difference in the microbial community to be visualized by the nMDS analysis. In contrast, a study in which the intestinal microbiota of rats treated with Li_2CO_3 and control rats was analyzed, there was a difference in the grouping of animals that received Li from animals that did not (Cussotto et al., 2019), which were treated for 28 days.

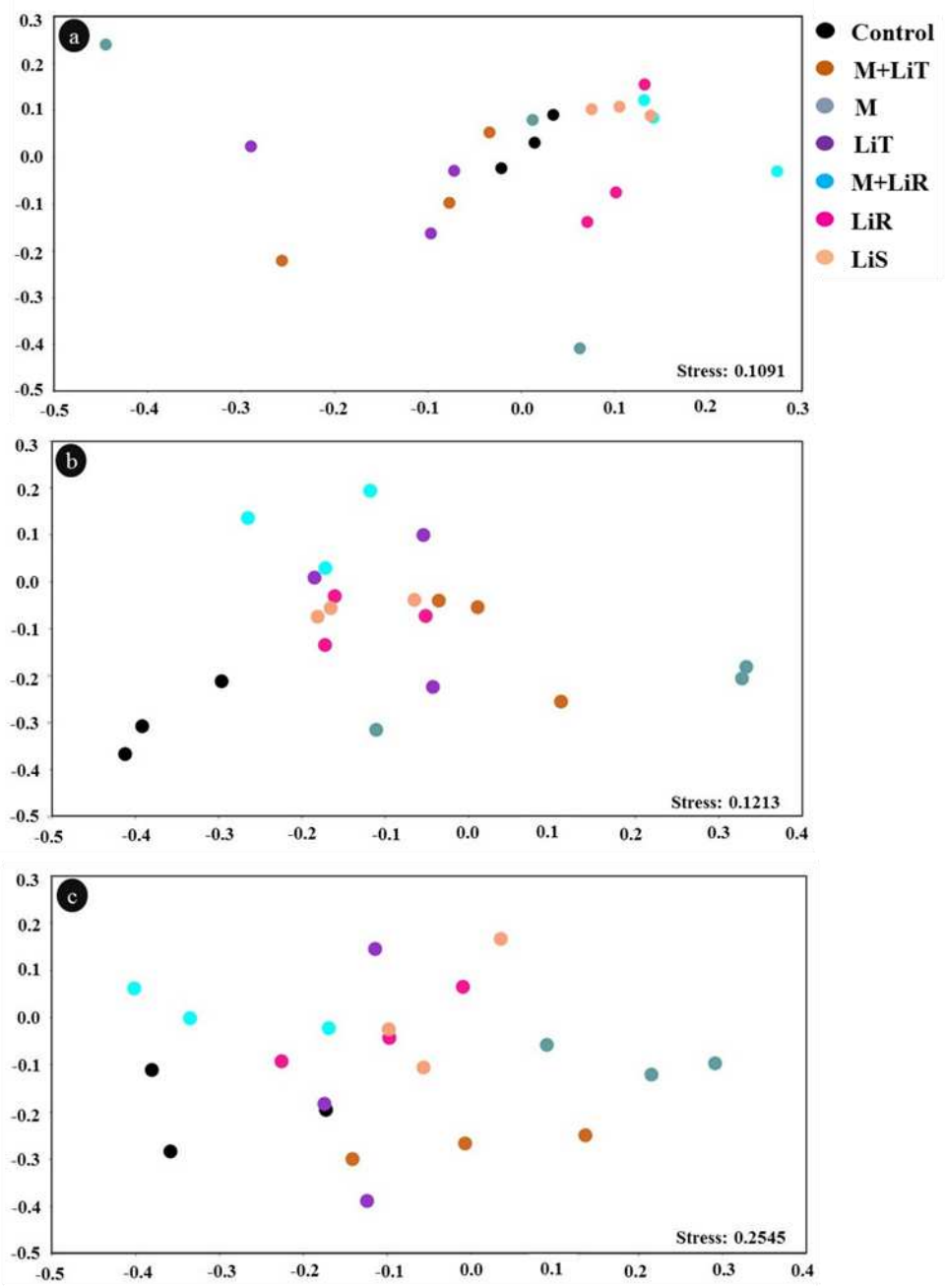

Figure 1. Non-metric multidimensional scale (nMDS) plots of Bray-Curtis dissimilarity index for bacterial communities of ileum (a), colon (b) and feces (c). Control (feed only), mushroom flour enriched at the therapeutic dose (300 mg of Li_2CO_3) mixed with the feed (M+LiT), unenriched mushroom flour mixed with the feed (M), lithium carbonate in therapeutic dosage (300 mg of Li_2CO_3) supplied in capsules (LiT), enriched mushroom flour in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (M+LiR), lithium carbonate in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (LiR) and overdose of lithium carbonate (600 mg of Li_2CO_3) supplied in capsules (LiS).

Alpha diversity varied only in the colon and feces samples (Dunnet's test, $p < 0.05$). Simpson's diversity index showed maximum values observed in the communities of the treatments M+LiT, LiT and LiS and minimum values in the communities of the control and the treatments M, M+LiR and LiR (Table 2). As can be seen, therapeutic dosages (M+LiT and

LiT), regardless of the form (enriched mushroom or Li_2CO_3) and overdose (LiS) can cause an increase in colon microbial diversity in piglets. Both the mushroom (M) and the recommended dosages (M+LiR and LiR) did not influence the diversity. The Shannon diversity index in the colon showed the highest diversity values for all treatments that received Li (M+LiT, LiT, M+LiR, LiR, LiS), regardless of form and dose (Table 2).

Maximum values of Chao richness indices in the colon and feces were observed in the microbial communities of the M+LiT, M+LiR and LiT treatments, the two latter only in the colon. Here we see that Li influenced the species richness of the colon in the therapeutic dosages (M+LiT and LiT) and recommended dosage (M+LiR) and the species richness of the feces in the treatments (M+LiT) (Table 2). In some studies, with rats, it was found that treatments that were treated with Li (about 150 mg kg^{-1}) increased the diversity of the gut microbiota (Shannon and chao index) compared to the control that did not receive Li (Cussotto et al., 2019; Huang et al., 2022).

Table 2. Alpha diversity indices in the ileum, colon and feces of piglets.

Index	Treatments						
	Control	**M+LiT**	**M**	**LiT**	**M+LiR**	**LiR**	**LiS**
				Ileum			
Simpson 1-D	0.75	0.80	0.82	0.85	0.73	0.74	0.66
Shannon	2.65	2.72	2.80	2.90	2.80	2.62	2.35
Chao-1	472.50	536.25	384.33	389.40	617.91	702.90	548.25
				Colon			
Simpson 1-D	0.90b	0.98a	0.90b	0.98a	0.96b	0.96b	0.98a
Shannon	3.90b	5.36a	4.40b	5.15a	5.00a	5.94a	5.00a
Chao-1	707.50b	1133.75a	850.60b	1059.33a	1153.64a	921.30b	972.70b
				Feces			
Simpson1-D	0.95	0.99	0.95	0.97	0.97	0.97	0.97
Shannon	4.75	5.50	4.82	5.00	4.80	5.00	4.90
Chao-1	775.85b	1061.20a	904b	886b	871b	990.50b	880b

For each index, means followed by different letters on the same line differed at the 5% significance level determined by Dunnet's test.

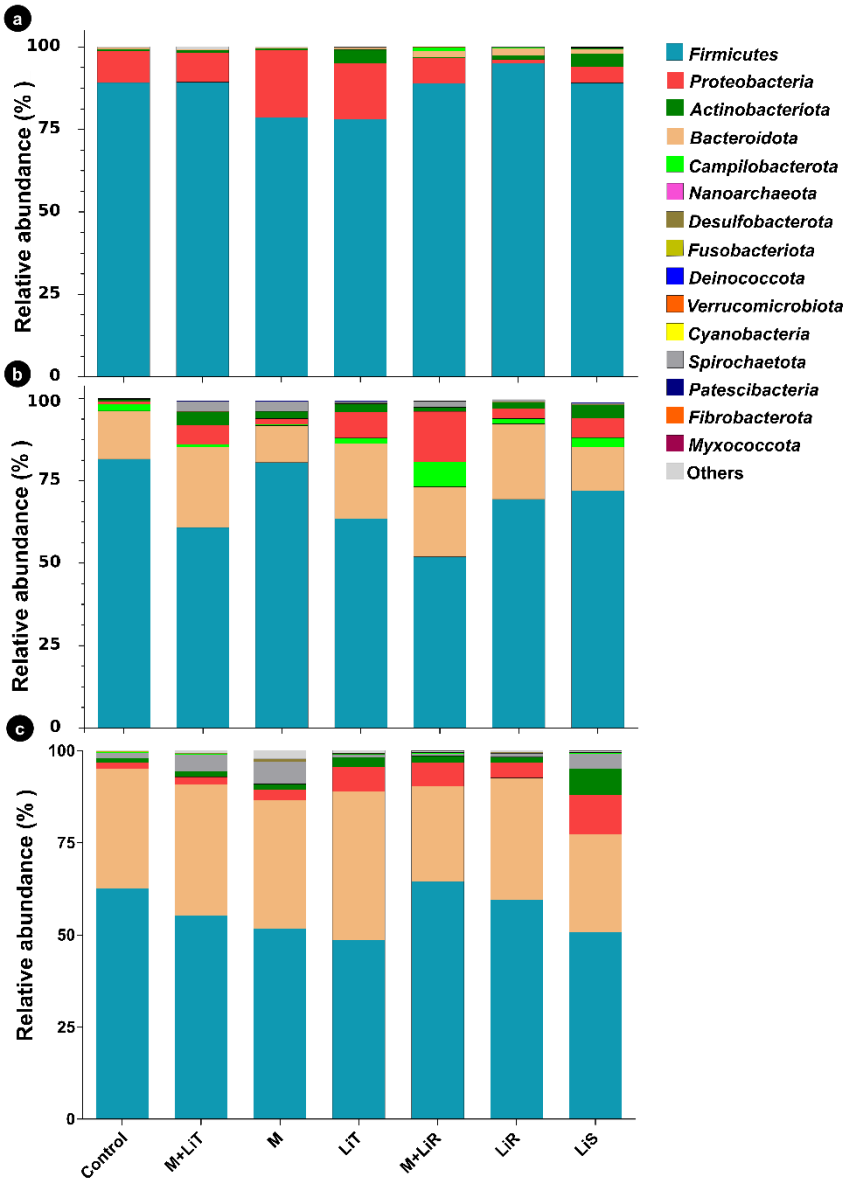

Figure 2. Phylum-level bacterial composition in ileum (a) colon (b) and feces (c) in piglets. Each bar represents the average composition of the bacterial community in the control (feed only), mushroom flour enriched at the therapeutic dose (300 mg of Li_2CO_3) mixed with the feed (M+LiT), unenriched mushroom flour mixed with the feed (M), lithium carbonate in therapeutic dosage (300 mg of Li_2CO_3) supplied in capsules (LiT), enriched mushroom flour in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (M+LiR), lithium carbonate in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (LiR) and overdose of lithium carbonate (600 mg of Li_2CO_3) supplied in capsules (LiS). We evaluated the 10 phyla with the highest relative frequencies. The other phyla are grouped under ‘’others.’’

Firmicutes was the predominant bacterial phylum in the ileum, colon and feces in all treatments, not differing between treatments ($p>0.05$) (Figure 2a). *Proteobacteria* and *actinobacteriota* were the other predominant phyla in the ileum microbial community (Figure 2a), except for the LiR treatment in which the phylum *Bacteroidota* had the second highest

relative frequency (2.09%) followed by *Actinobacteriota* (1.25%). In the colon, the predominant bacterial phyla were *Firmicutes* (81.61%) and *Bacteroidota* (14.54%) (Figure 2b).

In the feces, the most abundant phyla were also *Firmicutes* and *Bacteroidota*. This greater abundance of *Spirocaeota* in treatments M+LiT and M may be related to a greater proportion of fibers that were present mixed in the ration of these animals, since only these two treatments received 122 g of mushroom flour (Table 1) and this phylum is responsible for fiber degradation (Arora et al., 2022). There was no difference in bacterial composition between treatments at phylum level ($p>0.05$).

Our findings at the level of the most abundant phyla agree with works in the literature in which the most abundant phyla in piglets are *Firmicutes* and *Proteobacteria* in the ileum (Yang et al., 2016) and *Firmicutes* and *Bacteroidota* and *Proteobacteria* in fecal samples (Costa et al., 2014; Mach et al., 2015; Gardiner et al., 2020). In the literature, the phyla with the highest abundance in the colon were *Firmicutes*, *Proteobacteria* and *Bacteroidota* (Quan et al., 2018).

Some studies have shown a decrease in *Actinobacteriot*a abundance in individuals with some type of stress-related disorder (Reber et al., 2016; Malan-Muller et al., 2018). In our study, we found that *actinobacteriota* were among the 10 phyla with the highest relative abundance in the ileum, colon and feces and that in the M+LiT and LiS treatments, the colon is where the greatest abundance of this phylum occurs, differing both from the other treatments ($p< 0.05$). This is very interesting because both M+LiT and LiS treatments can enrich this group of microorganisms in the gut of individuals with some type of stress-related depression. In a study with rats treated with Li_2CO_3, it was verified an increase in the *actinobacteriota* phylum when compared to animals that did not receive Li_2CO_3 (Cussotto et al., 2019). This is an interesting finding since M+LiT corresponds to a therapeutic dosage of Li, and this dosage in the form of an enriched mushroom seems to favor the enrichment of this phylum in the colon of swine, differing even from the LiT treatment that have the same dosage of Li, but in the form of Li_2CO_3.

At the family level, *Lactobacillaceae* was the most representative in the ileum (figure 3a) and did not differ between treatments. The second most representative were the families *Enterobacteriaceae* (control, M and LiT), *Streptococcaceae* (M+liT and M+LiR), *Lachnospiraceae* (LiR) and *Pasteurellaceae* (LiS) (Figure 3a). In the ileum, *Peptostreptococcaceae* had a relative frequency almost 7 times higher in the M treatment compared to the other treatments with the highest frequencies ($p<0.05$) not only differing from the control, M+LiT, LiT and M. *Peptostreptococcaceae* is genuslly considered as a normal commensal bacterium, and its proportion is higher in the gut microbiota of healthy animals than

those with some gut microbiota dysbiosis, indicating that *Peptostreptococcaceae* may help maintain intestinal homeostasis (Leng et al., 2016; Fan et al., 2017). Increase in was related to the introduction of *Ganoderma lucidum* in the diet of rats (Diling et al., 2020). This increase in the population of this family in the M treatment in piglets may be associated with the various benefits associated with the consumption of mushrooms how such as stimulating the biosynthesis of tryptophan which is one of the essential amino acids, with antioxidant effects, as well as a precursor of the neurotransmitter serotonin, a sedative drug that regulates the circadian rhythm and improves sleep (Diling et al., 2020).

In genusl, in the colon, the families with the highest relative frequency were *Lactobacillaceae*, *Lachnospiraceae* and *Prevotellaceae*, which are responsible for the degradation of carbohydrates and proteins, in which the penultimate had greater abundance in the treatment that had mushroom flour added to the ration (treatment M). Addition of mushroom to the diet of rats led to the enrichment of intestinal *Lachnospiraceae* (Li et al., 2021). Although members of *Lachnospiraceae* are among the main producers of short-chain fatty acids, different taxa of *Lachnospiraceae* are also associated with different intestinal dysbiosis in humans (Vacca et al., 2020). The increased abundance of *Lachnospiraceae* may also be associated with a decrease in swine pathogens such as *Clostridium difficile* (Umu et al., 2015). As we can see the impact of *Lachnospiraceae* on host physiology is often inconsistent across different studies. In colon treatment M, the *Oscillospiraceae* family had a higher abundance when compared to the other treatments, but did not differ from them ($p>0.05$) (relative frequency of 4.5%) (Figure 3b). Some members of the *Oscillospiraceae* family are beneficial to intestinal health due to the production of butyrate that can be used as an energy source by the host, being a strong candidate as a probiotic (Yang et al., 2021).

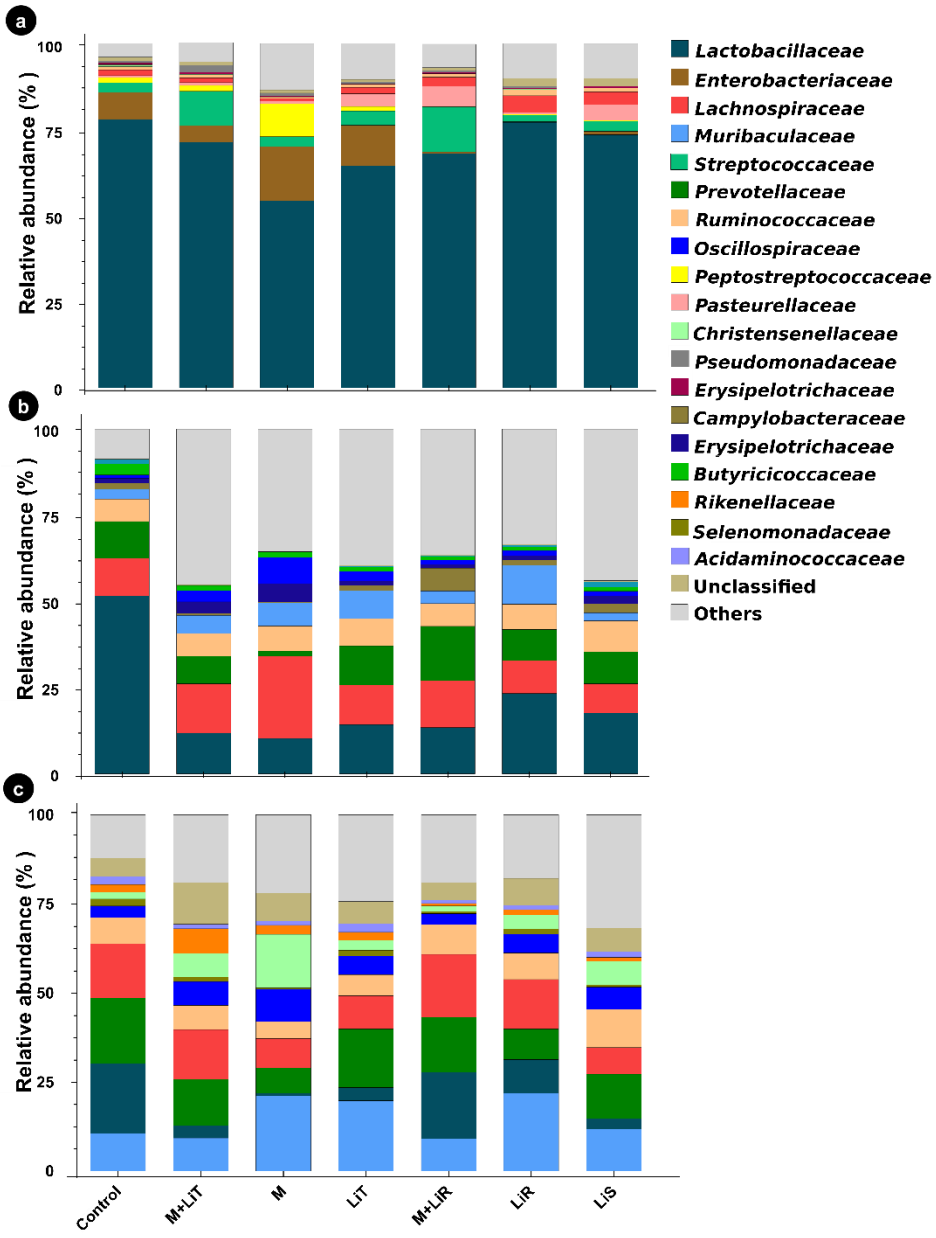

Figure 3. Family-level bacterial composition in ileum (a) colon (b) and feces (c) in piglets. Each bar represents the average composition of the bacterial community in the control (feed only), mushroom flour enriched at the therapeutic dose (300 mg of Li_2CO_3) mixed with the feed (M+LiT), unenriched mushroom flour mixed with the feed (M), lithium carbonate in therapeutic dosage (300 mg of Li_2CO_3) supplied in capsules (LiT), enriched mushroom flour in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (M+LiR), lithium carbonate in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (LiR) and overdose of lithium carbonate (600 mg of Li_2CO_3) supplied in capsules (LiS). We evaluated the 10 family with the highest relative frequencies.

In feces, the most representative family was *Muribaculaceae*, *Lactobacillaceae*, *Prevotellaceae, Lachnospiraceae*, *Ruminococcaceae* (Figure 3C). The microbial community of *Lactobacillaceae* varied among the treatments in which the animals that received the lowest doses of Li (1 mg) both in the form of Li_2CO_3 (LiR) and in the enriched mushroom form (M+LiR) were the only ones that did not differ from the microbial community of

Lactobacillaceae control ($p<0.05$). The *Lactobacillaceae* family is known to positively influence host health and has proven effects on intestinal permeability and the immune system and inhibit the growth of harmful bacteria (Oscarsson et al., 2021). This may indicate that both the mushroom and therapeutic dosages or Li overdoses can influence the composition of fecal *Lactobacillaceae* in piglets. The *Rikenellaceae* family had its frequency about 3 times higher in the feces of treatment M when compared to the other treatments ($p>0.05$). This family is considered to be protectors of cardiovascular and metabolic diseases as well as markers of healthy aging in humans (Tavella et al., 2021).

The *Muribaculaceae* family was detected only in the feces and colon of the animals (Figure 3C). This family is known to degrade a variety of complex carbohydrates (Lagkouvardos et al., 2019). *Muribaculaceae* and *Prevotellaceae* together represented the families with the highest abundance in the colon of healthy piglets (Tang, et al, 2020).

The 10 bacterial genus with the highest relative abundance in the ileum, colon and feces are represented in Figure 4. The predominant group in the ileum were *Lactobacillus*, *Escherichia-Shigella* and *Streptococcus* (Figure 4a). In the colon, *Lactobacillus* was also one of the most abundant, followed by the genus *Prevotella* and *Subdoligranulum* (Figure 4b). In the feces we had the predominance of the *Muribaculaceae*, *Prevotella* and *Lactobacillus* (Figure 4c). Some studies that sought to identify the profile of the microbial community in pig feces found that *Lactobacillus* (20.95%), *Prevotella* (6.41%), *Treponema* (2.48%), *Oscillospira* (1.96%), *Clostridium* (1.69%), *Ruminococcus* (1.36%), *Holdemania* (1.28%), *Streptococcus* (1.15%), *Bacteroides* (1.06%) and *Coprococcus* (0.87%) were among the top 10 genus of greater relative abundance, which represented the gut bacteria of different breeds of piglets (Xiao et al., 2016; Yang et al., 2018; Wang et al., 2022). These results suggest that some bacteria such as *Prevotella, Ruminococcus, Lactobacillus* and *Clostridium* are relatively constant in the intestinal microbiota of piglets and that these variations in diversity are linked to factors such as age, diet, sex, antibiotic use (Wang et al., 2022).

Within the genus *Lactobacillus*, only in the feces there was a difference between treatments ($p<0.05$) (Figure 4c). The treatments M+LiT, M, LiT and LiS differed from the others. Apparently, therapeutic dosages of Li, regardless of the form (enriched mushroom or Li_2CO_3), mushroom meal (M) and Li overdose (LiS) can cause a decrease in the *Lactobacillus* population in the intestine of piglets according to fecal samples. We can see this same trend in the colon, but there was no difference between treatments (Figure 4b).

The *Lactobacillus* genus, as well as *Streptococcus*, are an important member of the intestinal microbiota as they metabolize carbohydrates producing lactic acid that can be used

as an energy source by the host, in addition to being involved in immune, metabolic and intestinal homeostasis functions (Dempsey and Corr, 2022). There are studies with rats demonstrating that the abundance of *Lactobacillus* in the intestine is related to beneficial effects on anxiety and depression-like behaviors in addition to improving the intestinal barrier against pathogens and toxins (Malan-Muller et al., 2018; Peirce and Alviña, 2019).

Although the overgrowth of *Escherichia-Shigella* is usually associated with dysbiosis leading animals to diarrhea, in our work we did not verify this symptom in animals (Menezes-Garcia et al., 2020; Luo et al., 2022), except in the LiS treatment. Despite being associated with many diseases, *Escherichia-Shigella* are not exclusively pathogenic, being part of the natural intestinal microbial community of animals such as piglets and humans where they establish mutualistic relationships that maintain intestinal homeostasis (Martinson and Walk, 2020).

Prevotella is among the 10 most abundant genus in the colon and feces of piglets, not differing statistically between treatments. This genus has the ability to digest complex carbohydrates and genetic and enzymatic potential to break down fiber of plant origin, being present in greater abundance in populations with this diet when compared to diets rich in protein and fat (Precup and Vodnar, 2019). There are studies showing that decreased abundance of the *Prevotella* genus was associated with depressed individuals (Wingfield et al., 2021) and/or with some other illness related to mood disorder when this genus was compared to the control (Tomizawa et al., 2021). Contrary to the benefits of these groups in minimizing the symptoms of mood-related diseases, there are studies relating the abundance of the *Faecalibacterium* genus to patients with depression (Jiang et al, 2015; Chang et al., 2021). This genus was among the 10 genus with the highest relative abundance only in the colon and feces and there was no difference in abundance between treatments. Apparently lithium, regardless of the way it was administered to animals, does not influence the composition of the *Faecalibacterium* community.

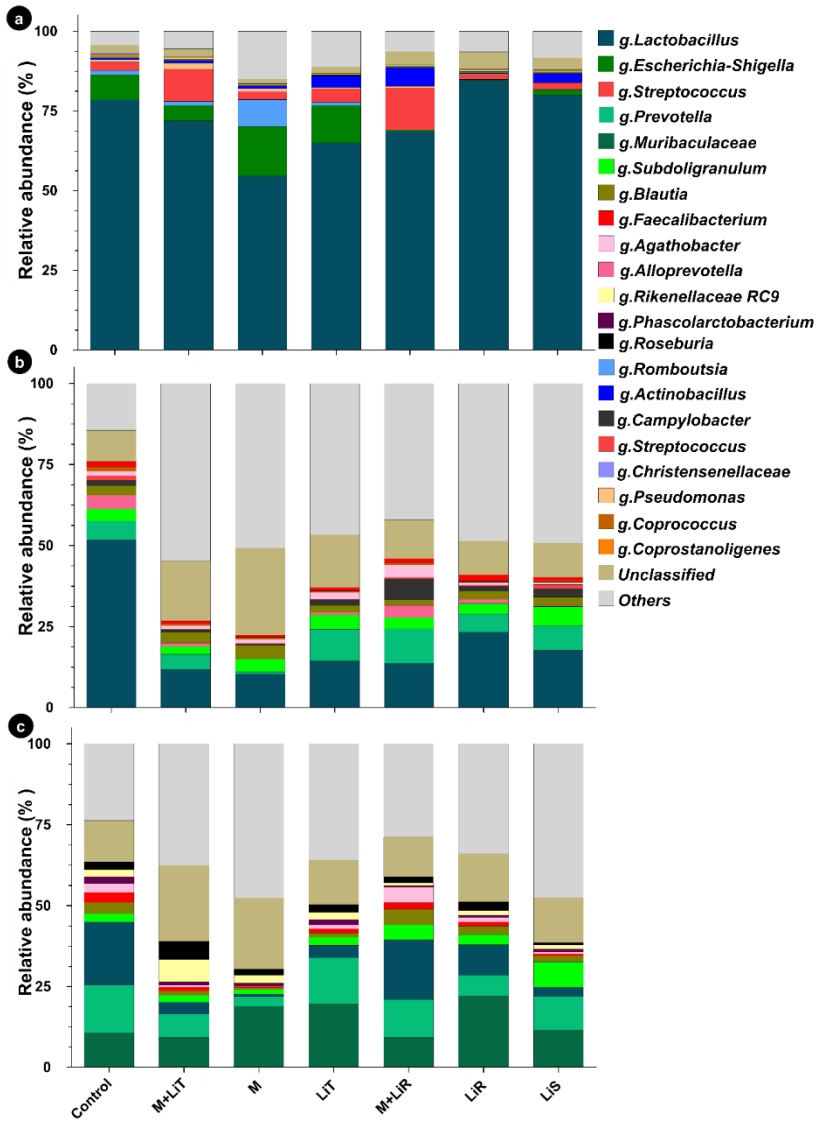

Figure 4. Gender-level bacterial composition in ileum (a) colon (b) and feces (c) in piglets. Each bar represents the average composition of the bacterial community in the control (feed only), mushroom flour enriched at the therapeutic dose (300 mg of Li_2CO_3) mixed with the feed (M+LiT), unenriched mushroom flour mixed with the feed (M), lithium carbonate in therapeutic dosage (300 mg of Li_2CO_3) supplied in capsules (LiT), enriched mushroom flour in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (M+LiR), lithium carbonate in the recommended dosage (1 mg of Li_2CO_3) supplied in capsules (LiR) and overdose of lithium carbonate (600 mg of Li_2CO_3) supplied in capsules (LiS). We evaluated the 10 family with the highest relative frequencies. We evaluated the 10 genus with the highest relative frequencies. The other genus are grouped under "others".

Muribaculaceae was the group with the highest relative abundance in feces. This genus is a recently discovered group of bacteria commonly found in the gastrointestinal tract of mammals that act in the fermentation of complex polysaccharides, being one of the most important groups that produce propionate (Smith et al., 2021). The LiS treatment animals (which received 600 mg of Li_2CO_3) showed symptoms of intoxication such as tremors, diarrhea and loss of appetite. Diarrhea is caused by bacterial pathogens such as *Escherichia, Shigella*,

Salmonella, *Campylobacter*, *Clostridium difficile* and *Aeromonas* (Li et al., 2021). In our work, no members of the genus *Salmonella* or *Aeromonas* were found, but *Escherichia-Shigella*, *Campylobacter* and *Clostridium* were present. The last two (despite not being among the 10 with the highest abundance) had higher relative abundance in the ileum in the LiS treatment, but did not differ statistically from the other treatments. It may be that there was not enough time for the microbiota to undergo a noticeable change as the animals were treated with Li_2CO_3 only for 5 days.

To filter the differences between bacterial communities between treatments and between the ileum, colon and feces we used Venn diagrams to analyze ASVs that were shared (Figure 5). We found 19.339 ASVs distributed in all samples, being 4.319 exclusive to the ileum, 5.162 to the colon and 6.343 to the feces. We found that 1.262 ASVs are shared between the ileum, colon and feces (Figure 5a). The largest number of ASVs sampled was in the feces.

We constructed Venn diagrams only with the treatments M+LiT (56 mg of Li in the form of enriched mushroom) and LiT (Li in the form of Li_2CO_3) to verify if there was a change in the number of ASVs depending on the source of Li that was supplied to the patient's animals (Figure 5i-k). In all analyzed areas and feces, the number of ASVs was higher in the M+LiT treatment (Figure 5i-k) when compared to LiT, especially in the ileum where the difference is almost double (Figure 5i). We did this analysis because there is an *in vivo* study with piglets that showed (unpublished data) that Li in the mushroom form was more bioavailable and genusted less free radicals when compared to its Li_2CO_3 form. In view of these results, we can infer that the enriched mushroom is a source of Li that favors a greater diversity of microorganisms in the gastrointestinal tract of piglets when compared to Li_2CO_3.

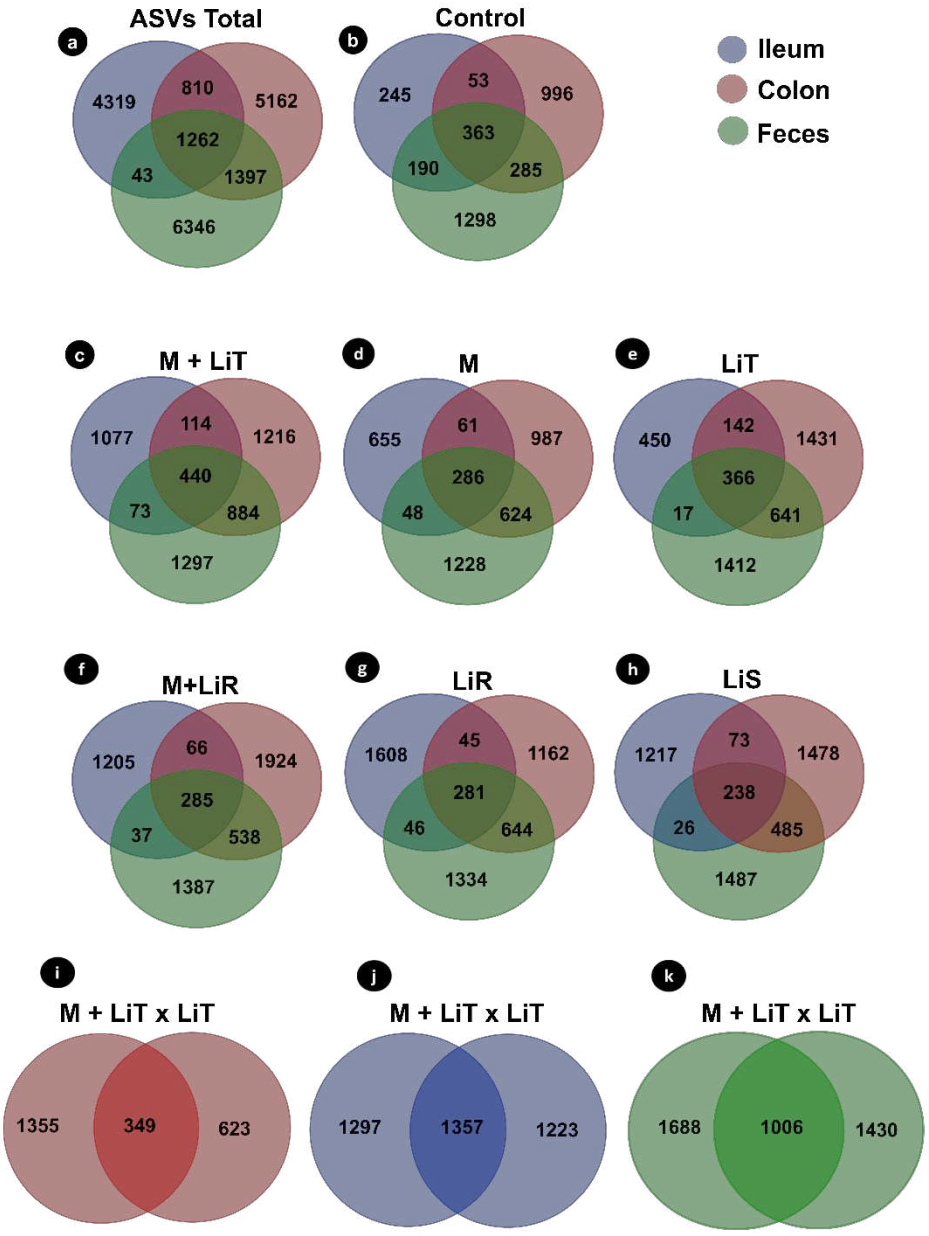

Figure 5. Venn diagrams showing the number of bacterial ASVs. (a) total ASVs shared between the ileum, colon and feces; (b) ASVs from the control group shared between the ileum, colon and feces; (c) M+LiT treatment ASVs shared between ileum, colon and feces; (d) M-treatment ASVs shared between ileum, colon and feces; (e) ASVs from LiT treatment shared between ileum, colon and feces; (f) M+LiR treatment ASVs shared between ileum, colon and feces; (d) ASVs from LiR treatment shared between ileum, colon and feces; (g) ASVs from LiS treatment shared between ileum, colon and feces; (h) M-treatment ASVs shared between ileum, colon and feces; (i) Comparison of the number of ASVs from M+LiT and LiT treatments in the ileum; (j) Comparison of the number of ASVs from M+LiT and LiT treatments in the colon; (k) Comparison of the number of ASVs from M+LiT and LiT treatments in feces.

LEfSe and LDA analysis based on ASVs

To verify which treatments enriched the intestinal microbiota of piglets, we performed LEfSe and LDA analysis (Figure 7), where we can have observed some genus showed differential abundance between treatments ($p<0.05$).

The LDA scores indicated that the relative abundances of genus such as *Enterorhabdus, Muribaculaceae* (ileum), *Ruminococcaceae* (colon*), Lactobacillus* and *Coprococcus* (feces) were more enriched in the M+LiR treatment (mushroom enriched with the recommended dosage). Studies have shown a lower abundance of these genus in rats and humans with some mood disorder, anxiety and/or depression compared to the control group (Burokas et al., 2017; Heym et al., 2019; Wang et al., 2020; Zheng et al., 2021; Zou et al., 2021).

In the LiS treatment, genus such as *Weissella* and *Olsenella* were enriched in the colon. As already mentioned in this treatment, the animals presented diarrhea. These genus are not associated with diarrhea, on the contrary, there is work showing a decrease in *Weissella* in rats with diarrhea (Zhuang et al., 2018). In the M+LiT treatment, there was enrichment of *Prevotellaceae* in the colon and feces. Decline in *Prevotellaceae* is associated with anxiety disorders in humans (Chen et al., 2019). There was enrichment of *Bacteoidales*, *Ruminococcus* and *Clostridia* in the treatment (LiT) in colon and feces respectively. *Bacteoidales* and *Clostridia* groups are associated with depressed individuals and healthy individuals, respectively (Liu et al., 2020). Decrease in the *Ruminococcus* population was associated with human patients with depression and bipolar disorder in addition to being a genus with low richness in individuals with inflammatory bowel diseases leading to diarrhea (Liu et al., 2020; Daliri et al., 2020; Zou et al., 2021).

In the feces, there was *Burkholderia* enrichment in which its low and high abundance was found in depressed rats and humans with depression, respectively (Chen et al., 2019). *Bacteoidales, Micrococcaceae* had their relative abundance increased in humans with depression when compared to healthy humans (Liu et al.,2020; Fontana et al.,2020).

Figure 7. The LEfSe analysis showed different taxa abundant as biomarkers depending on the treatment for piglets by the Kruskal-Wallis test ($p < 0.05$). Taxa abundant as biomarkers in the ileum (a), Colum (b) and feces (c).

Here we relate the increase in the relative abundance of some groups of microorganisms that are considered as biomarkers in some psychiatric diseases. Studies have shown that altering the structure and composition of the intestinal microbiota affects the development of diseases since this microbiota produces metabolites that can act directly on the central and peripheral nervous system (Zou et al., 2021). As we could see some important groups such as *Lactobacillus* and *Ruminococcus* were in greater relative abundance ($p<0.05$) in treatments that received Li, showing that this element can have its therapeutic effects through the modulation of the intestinal microbiota and that mushrooms enriched with this element can be an alternative source of Li enriching certain beneficial groups of microorganisms such as *Lactobacillus* which had their relative abundance increased only in the M+LiR treatment.

Production of short chain organic acids (SCOA)

The Table 2 list the main short-chain organic acids found in piglets from each treatment. As we can see, there was a difference in the production of short-chain organic acids between some treatments ($p<0.05$). This variation is related to the relative abundance of some groups of microorganisms in the gastrointestinal tract of piglets.

We can make a relationship between the results of the abundance of the microbial community and the production of organic acids by these microorganisms. The production of lactic acid, for example, is directly related to the abundance of *Lactobacullus* (major producers of lactic acid) in the feces, in which the treatments M+LiT, M, LiT and LiS were the ones that showed the lowest values for both the abundance of this genus and the production of lactic acid (Table 2). Li in therapeutic dosages, regardless of the source, seems to influence the production of these acids in piglets. The treatment that received only mushroom flour also had reduced lactic acid production. This may be related to the type of substrate that is degraded by *Lactobacillus*, in which this genus is known to degrade carbohydrates (Dempsey and Corr, 2022) and this treatment (M) had mushroom flour added which is rich in fiber.

The therapeutic level of Li dosages, regardless of the form (unenriched mushroom or enriched mushroom or Li_2CO_3) and overdose (LiS) decrease the production of these organic acids when compared to the control. In a study with rats, Li increased the production of propionate, butyrate and acetate acids and the biosynthesis pathways of organic acids was upregulated after treatment with this element (Cussotto et al., 2019; Huang et al., 2022). Another hypothesis is that Li at these dosages can increase the absorption of these acids by the host and thus less acid is quantified in the analyses. The other acids such as acetic, propionic and butyric are quite widespread within the phyla *Firmicutes* and *Bacteroidetes* (Venkataraman

et al., 2016). The family *Lachnospiraceae* and *Ruminococcaceae* have received more attention because they are very abundant in the human colon, comprising 10 to 20% of the total bacteria and produce butyric acid (Vital et al., 2014). These acids can improve gut health through a number of local effects, ranging from maintaining intestinal barrier integrity, producing mucus and protecting against inflammation to reducing the risk of colorectal cancer. (Silva et al., 2020). In piglets the energy contribution of Short-chain organic acids to the basal metabolic rate is estimated to be 10-30% (Bergman, 1990; Rhouma et al., 2021).

Table 2. Profile of organic acids (SCOA) in pig feces according to the experimental groups

SCOA (g L^{-1})	**Treatments**						
	Control	**M+LiT**	**M**	**LiT**	**M+LiR**	**LiR**	**LiS**
Acetic	0.55a	0.32	0.10	0.34	0.37a	0.38a	0.33
Propionic	0.25a	0.13	0.05	0.14	0.17a	0.18a	0.13
Butyric	0.16a	0.10	0.03	0.10	0.13a	0.13a	0.10
Lactic	0.11a	0.04	0.01	0.04	0.04	0.06a	0.02
Total SCOA	1.07a	0.57	0.19	0.62	0.72a	0.75a	0.58

Means not labeled with the letter a are significantly different from the treatment mean at 5% significance by Dunnet's test.

CONCLUSIONS

This is the first work that shows the effects not only of Li, but also of Li-enriched mushrooms on the composition of microbial communities in the swine ileum, colon and feces. We found that Li can influence the abundance and richness of some genus of bacteria in the intestines of these animals. Li at the recommended dosage (enriched mushroom) and therapeutic (enriched mushroom and Li_2CO_3) can exert its effects via population modulation of some specific groups of microorganisms, such as *Lactobacillus* spp. and *Ruminococcus* spp., in which the richness of these genus is associated with healthy individuals. It is noted that the overdose was poorly enriched with few genus and these are little exemplified in the literature as being important for the intestinal homeostasis of the host. Studies for a longer period of time should be carried out so that we can verify these changes in microbial composition.

Acknowledgments

The authors are grateful to the Cordenação de Aperfeiçoamento de Pessoal de Nível superior (Capes-001), Conselho Nacional de Desenvolvimento Científico e Tecnológico

(CNPq) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG) for their financial support.

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CONCLUSÕES GERAIS

O presente trabalho contribui para o aprofundamento do conhecimento em enriquecimento de cogumelos com lítio. *Pleurotus djamor* e *Pleurotus ostreatus* são bons acumuladores de lítio em seus cogumelos, sendo que o primeiro é um dos fungos que mais bioacumula lítio, sem perda na produtividade. Além disso, ocorre o co-acúmulo de outros minerais concomitantes ao acúmulo de lítio. O lítio é mais bioacessível, em ambos os cogumelos, quando comparado ao medicamento carbonato de lítio. Demosntramos pela primeira vez a biodisponibilidade de cogumelos enriquecidos com lítio *in vivo*. O lítio presente em cogumelo de *P, djamor* enriquecidos é mais biodisponível do que o carbonato de lítio. Além disso, os animais apresentaram menos danos oxidativos, a depender do tecido, quando a fonte de lítio era o cogumelo enriquecido quando comparado ao carbonato de lítio.

Nosso trabalho fornece também um estudo dos efeitos do lítio e cogumelos enriquecidos na composição da microbiota intestinal de suínos. Verificamos que alguns grupos de microorganismos importantes para a saúde do hospedeiro, como *Lactobacillus* e *Ruminococcus,* tem suas populações aumentadas em animais que foram tratados com cogumelo enriquecido com lítio. Os fungos bioacumuladores de minerais, como o *P. djamor* e *P. ostreatus*, apresentam potencial para serem utilizados na saúde humana e animal, pois além de aumentar a disponibilidade de Li, diminui os efeitos tóxicos em indivíduos que fazem o seu uso terapêutico e aumentando populações de microorganismos importantes que mantêm a homeostase do intestino.