



IMPACT OF THE MICROELEMENTS SUPPLEMENTED IN THE CASING LAYER ON THE CHEMICAL CONSTITUENTS OF THE EDIBLE MUSHROOM *AGARICUS BISPORUS*

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Article history:	Abstract:
Received: 14 th August 2023	The experiment was conducted at the mushroom farm in the College of Agriculture, University of Tikrit. Four microelements, namely iron, zinc, manganese, and molybdenum, were tested in three concentrations of 50, 100, and 200 mg L ⁻¹ , in addition to a control treatment. All of these were added to the casing soil of the edible mushroom <i>Agaricus bisporus</i> . The results indicated that the Mo ₁₀₀ (molybdenum at 100 mg L ⁻¹) treatment yielded the highest protein and ash contents in the mushroom fruit bodies, reaching 28.31% and 13.23%, respectively, with an increase percentage over the control treatment of 26.60% and 14.29% for protein and ash, respectively. The Mn ₅₀ (manganese at 50 mg L ⁻¹) and control treatments superior on other treatments in the total carbohydrate content of the mushroom fruit bodies, with percentages of 66.29% and 65.38%, respectively. The Mix ₁₀₀ (microelements mixture at 100 mg L ⁻¹) treatment recorded the highest dry matter content, with a 21.94% increase over the control treatment. As for total fat content, the Mn ₁₀₀ and Mo ₁₀₀ superior on other treatments compared with the lowest fat content in the control. The Zn ₁₀₀ exhibited a superior raw fiber content in the fruit bodies by 19.80%. Meanwhile, the Mn ₁₀₀ recorded the highest caloric value reaching to 382.80 calories 100 g ⁻¹ of dry weight.
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INTRODUCTION

The edible mushroom *A. bisporus* is considered a delicious food, rich in nutritional value, and has been incorporated into various food products. It can be consumed cooked, fried, grilled, or fermented. This mushroom contains well-balanced nutritional components, with a significant emphasis on protein, which contains all essential amino acids. In terms of nutritional value, its protein content falls between vegetable and meat proteins, ranging from 19% to 35% of dry weight (Wang and Zhao, 2023). Mushrooms contain high concentrations of vitamins such as ascorbic acid (vitamin C), thiamine, riboflavin, niacin, pyridoxine, and cobalamin (vitamin B12). They also contain small amounts of fat-soluble vitamins like A and E. Mushrooms are the only non-animal source of vitamin D, which is of particular importance to individuals following a vegetarian diet. Vitamin D plays a crucial role in the health of bones, cartilage, and teeth (Ayimbila et al., 2023).

Fats are present in small quantities in the fruit bodies, mostly in the form of unsaturated fats, constituting approximately 79.7% of the total fat content. Linoleic acid, an essential component of the human diet, is a major constituent of these fats, this acid plays a crucial role in treating various diseases, including cardiovascular diseases, reducing triglyceride levels, managing high blood pressure, and alleviating inflammation (Golak-Siwulska et al., 2018). Carbohydrates make up the majority of *A. bisporus* nutritional composition, averaging around 53% of dry weight. They are primarily found in the form of complex carbohydrates, including glycoproteins, chitin, polysaccharides, α -glucans, β -glucans, xylans, and galactans (Golak-Siwulska et al., 2018). Mannitol constitutes the major portion of sugars in the mushroom, along with glucose, fructose, galactose, trehalose, and mannose (Tseng and Mau, 1999). The fruit bodies also contain a variety of essential minerals, including potassium, phosphorus, magnesium, calcium, and sodium. Additionally, they contain trace elements such as iron, copper, zinc, and manganese. Selenium is another important trace element found in mushrooms, playing a significant role in enhancing the immune system (Aboubakr et al., 2018).

Fungi, like all living organisms, require a range of nutrients to derive energy and materials for building their structures and reproductive processes, as well as to maintain their vitality. Among these nutrients are trace minerals, which fungi need in small quantities to meet their minimum essential requirements. Trace elements play a crucial role in various biological processes of fungi, primarily as cofactors for enzymes. Some of the most important trace minerals for fungi

include zinc, copper, manganese, iron, selenium, and molybdenum (Sherif, 2012; Robinson et al., 2021). Weil et al. (2006) conducted a study to investigate the impact of Micromax fertilizer, composed of a mixture of various trace elements, on the yield of the *A. bisporus* mushroom. The results showed an overall increase in yield, although the specific elements responsible for this effect were not identified. However, when manganese was added in conjunction with Micromax, a yield increase ranging from 9.6% to 11.8% was achieved. The study suggested that manganese was likely responsible for this increase.

In a study conducted by Royse and Estrada (2007), an increase in yield, vitality, and the size of fruit bodies of the *Pleurotus eryngii* mushroom was observed after adding 50 $\mu\text{g gr}^{-1}$ of manganese. However, higher concentrations of manganese led to an increase in the protein, potassium, phosphorus, boron, and zinc content of the fruit bodies, while the levels of magnesium, manganese, aluminum, and sodium in the fruit bodies were not affected. Maknali et al. (2021) found that the casing layer supplemented with iron led to an increase in the protein content of the fruit bodies compared to the control. Additionally, Yokota et al. (2016) reported that adding iron to the cultivation medium of oyster mushrooms increased the protein content in the fruit bodies by 10.4 and 6.6%, respectively. Given the limited number of studies in this area, this study was conducted to investigate the impact of adding trace elements during the casing layer phase on the chemical constituents of the *A. bisporus* edible mushroom.

MATERIALS AND METHODS

The study was conducted at the mushroom farm affiliated with the College of Agriculture at the University of Tikrit during the mushroom production cycle from November 5, 2020, to December 4, 2021, for investigate the impact of adding microelements to the casing layer on the chemical constituents of the edible mushroom *A. bisporus*.

Production of *A. bisporus* fruit bodies

The preparation of *A. bisporus* (A_{15}) spawn, compost, casing soil and all necessary steps for fruit body production were implemented following the standard method employed in previous studies (Stamets, 2000; Hassan et al., 2002; Oei, 2005).

Application of microelements

After full growth compost with mushroom mycelium, 5 kg of casing soil per experimental unit, with a height of 4 cm, were applied. The required concentrations of microelements were prepared in 250 ml of distilled water and sprayed onto the casing soil after 3 days.

The experimental factors

The experimental factors included the addition of four trace elements: Fe, Zn, Mn, and Mo at three different concentrations, as well as a mixture of these elements (1:1:1:1, v:v) and the control treatment, resulting in a total of 16 treatments:

1. Fe₅₀ (Iron at 50 mg L⁻¹)
2. Fe₁₀₀ (Iron at 100 mg L⁻¹)
3. Fe₂₀₀ (Iron at 200 mg L⁻¹)
4. Mn₅₀ (Manganese at 50 mg L⁻¹)
5. Mn₁₀₀ (Manganese at 100 mg L⁻¹)
6. Mn₂₀₀ (Manganese at 200 mg L⁻¹)
7. Zn₅₀ (Zinc at 50 mg L⁻¹)
8. Zn₁₀₀ (Zinc at 100 mg L⁻¹)
9. Zn₂₀₀ (Zinc at 200 mg L⁻¹)
10. Mo₅₀ (Molybdenum at 50 mg L⁻¹)
11. Mo₁₀₀ (Molybdenum at 100 mg L⁻¹)
12. Mo₂₀₀ (Molybdenum at 200 mg L⁻¹)
13. Mix₅₀ (Microelements mixture of Fe, Zn, Mn, and Mo at 50 mg L⁻¹)
14. Mix₁₀₀ (Microelements mixture at 100 mg L⁻¹)
15. Mix₂₀₀ (Microelements mixture at 200 mg L⁻¹)
16. Control (spray with water only)

Studied characteristics

The studied characteristics included:

Protein (%)

Protein content was determined by estimating the percentage of nitrogen using the Micro kjeldahl method (Jackson, 1958), then calculated using the equation: Protein (%) on a Dry Weight Basis = Nitrogen (%) \times 6.25

Ash (%)

Ash content in the mushroom fruit body powder was determined according to the method outlined in A.O.A.C. (1995).

Fat (%)

Fat (%) was determined using a Soxhlet apparatus following the method proposed by A.O.A.C. (1995).

Crude Fiber (%)

Crude fiber was determined using the method mentioned in A.O.A.C. (1995).

Dry Matter (%)

A total of 100 g of fresh mushroom fruit bodies were taken for each treatment, cut into small pieces, and dried in an electric oven at a temperature of 60°C until a constant weight was achieved (Dundar et al., 2008). The percentage of dry matter was calculated using the following equation: % Dry Matter = (Dry Weight of Mushroom Fruit Bodies / Wet Weight of Mushroom Fruit Bodies) × 100

Total Carbohydrate (%)

Carbohydrates was calculated using the formula: Carbohydrates (%) = 100 - (Protein % + Fat % + Ash %), following the AOAC (1995) method.

Caloric Value (kcal 100 g⁻¹ of dry weight)

The caloric value was calculated using the formula: Caloric Value = 4 × (Protein % + Carbohydrates %) + 9 × Fat %, according to the AOAC (1995) method.

Statistical Analysis

The study experiments were conducted using a Completely Randomized Design (CRD), and the analysis of variance was performed using the Statistical Analysis System (SAS). Means were compared using Duncan's multiple range test at a significance level of 0.05 (Al-Rawi and Khalef Allah, 2000).

RESULTS AND DISCUSSION

Effect of the microelements supplemented in the casing layer on the protein, fats, ash, and carbohydrates of *Agaricus bisporus* fruit bodies

The results presented in Table 1 indicate that Mo₁₀₀ treatment significantly superior on the other treatments, recording the highest protein content in the fruit bodies at 28.31%, followed by Mo₅₀ and Mix₂₀₀ treatments with percentages of 26.80% and 26.68%, respectively. In contrast, the control treatment showed the lowest protein content at 20.78%. Mn₁₀₀ and Mo₁₀₀ treatments recorded the highest fruit bodies fat content reached to 4.34% and 4.25%, respectively, which were statistically superior to the other treatments, followed by 3.83% and 3.74% in the Mo₅₀ and Fe₁₀₀ treatments, respectively, compared with lowest fat content 2.50% in the control.

The results in table 1 also showed the Mo₁₀₀ recorded the highest ash content at 13.23%, followed by 12.69% in the Mix₁₀₀, while the Zn₂₀₀ showed the lowest ash content reaching 9.09%.

For the total carbohydrate content in the mushroom fruit bodies, it is evident from the results in the table 1 that Mn₅₀ and control treatments had the highest content, resulting in 66.29% and 65.38%, respectively, followed by 64.45% in the Fe₅₀ treatment, in contrast, the Mo₁₀₀ recorded the lowest carbohydrate content at 54.21%.

From the above results, we observe variations in the chemical components depending on the elements and their concentrations. There is an increase in protein content with increased supplementation of iron, which is consistent with the findings of Maknali et al. (2021), indicating that reinforcing the casing layer of *A. bisporus* with iron led to an increase in the protein content of the fruit bodies compared to the Control. Yokota et al. (2016) also found that adding iron to the cultivation medium of oyster mushrooms at 100 and 200 mg/kg increased the protein content in the fruit bodies by 10.4% and 6.6%, respectively, and confirmed a close correlation between the nitrogen content in the fruit bodies and the protein since the latter depends on the nitrogen content, which is a constituent of the amino acids that make up the protein. The results also indicate that low concentrations of zinc (50 mg/L) led to an increase in protein synthesis in the fruit bodies. This can be attributed to the role of zinc in protein synthesis, as explained by Paris and Jones (1997). Zinc is essential for the synthesis of proteins, carbohydrates, fats, nucleic acids, and polysaccharides. However, at higher concentrations, zinc can have negative effects due to the generation of free radicals, potentially leading to cell toxicity (Hänsch and Mendel, 2009). This result is consistent with Zn₁₀₀ and Zn₂₀₀, which resulted in a decreased protein content in the fruit bodies compared to Zn₅₀.

The results of the current study are somewhat consistent with Royse and Estrada (2007) and Elhami et al. (2008) in that the protein content in fruit bodies increased after supplementing the medium of the oyster mushroom *Pleurotus* spp. with Mn. This could be attributed to the role of Mn in stimulating enzymes, especially manganese peroxidase, which acts on lignin degradation and releases nutrients into the medium (Tien and Cai, 1987). A significant portion of these released nutrients includes nitrogen, which is directly involved in the synthesis of amino acids, the fundamental building blocks of proteins in fruit bodies. The increase of protein content in fruit bodies in the presence of molybdenum may be attributed to its prominent role in nitrate reduction and biological nitrogen fixation mediated by microorganisms, leading to an increase in the synthesis of amino acids, which are involved in protein production (Silke and Chantal, 2016). These results are consistent with the findings of Al-Daraji and Hassan (2022), who also observed an increase in protein content in *A. bisporus* fruit bodies with molybdenum supplementation. They also align with the results of Pirkani et al. (2019) regarding protein content in fruit bodies but differ from the results obtained by Wang et al. (2018) and Jacinto-Azevedo et al. (2021). It's noteworthy from the previous table that the increase in protein content in fruit bodies corresponds to a decrease in carbohydrate content, which is in agreement with previous studies conducted by Jurak et al. (2014) and Chang and Wasser (2017), but differs from the results of Zied et al. (2011).

Maknali et al. (2021) reported an increase in carbohydrate content in the fruit bodies of oyster mushrooms with iron supplementation at 500 mg L⁻¹. This finding aligns with the results obtained by Yokota et al. (2016), who observed that iron supplementation leads to an increase in ash content in the fruit bodies of oyster mushrooms, resulting in a 13.3% and 14.7% increase when iron was supplemented at 100 and 200 mg L⁻¹, respectively. These results are consistent

with previous studies on carbohydrate and fat content in fruit bodies (Jacinto et al., 2021) as well as ash content (Wang et al., 2018).

Table (1) Effect of the microelements supplemented in the casing layer on the protein, fats, ash, and carbohydrates of the edible mushroom *Agaricus bisporus* fruit bodies

Treatment	Chemical components (%)			
	Protein	Fats	Ash	Carbohydrates
Fe ₅₀	22.97 d	3.40 cd	9.18 f	64.45 bc
Fe ₁₀₀	26.63 b	3.74 bc	10.35 cd	59.28 g
Fe ₂₀₀	25.86 b	2.81 fg	10.17 c-e	61.17 ef
Mn ₅₀	20.51 e	3.57 bcd	9.63 d-f	66.29 a
Mn ₁₀₀	23.48 d	4.34 a	9.72 d-f	62.47 de
Mn ₂₀₀	25.36 c	2.55 g	9.81 d-f	62.28 de
Zn ₅₀	26.64 b	3.40 cd	9.27 ef	60.69 f
Zn ₁₀₀	23.95 d	2.72 fg	9.12 f	64.21 bc
Zn ₂₀₀	23.50 d	2.98 ef	9.09 f	64.44 bc
Mo ₅₀	26.80 b	3.83 b	10.35 cd	59.03 g
Mo ₁₀₀	28.31 a	4.25 a	13.23 a	54.21 h
Mo ₂₀₀	25.12 c	2.72 fg	11.07 bc	61.09 ef
Mix ₅₀	22.74 d	3.40 cd	10.44 cd	63.42 cd
Mix ₁₀₀	26.19 bc	3.20 de	12.69 a	57.92 g
Mix ₂₀₀	26.68 b	3.32 de	11.79 b	58.21 g
Control	20.78 e	2.50 g	11.34 b	65.38 ab

*Similar letters indicate that there are no significant differences according to Duncan's multiple test at the probability level of 0.05.

Effect of the microelements supplemented in the casing layer on the dry matter, crude fiber and calorie content of *Agaricus bisporus* fruit bodies

From the results presented in Table (2), it is evident that the Mix₁₀₀ treatment yielded the highest dry matter content in the fruit bodies, reaching 9.39%, which was a 2.98% increase compared to the Mo₂₀₀ treatment (9.11%) Furthermore, the Zn₁₀₀ treatment recorded the highest crude fiber content at 19.80%, significantly different from the other treatments with increase of 7.07 and 7.58% over the Mix₁₀₀ and Mn₂₀₀ treatments, respectively, while the lowest crude fiber content was 11.50% in the control. In calorie content of the *A. bisporus* fruit bodies, Mn₁₀₀ and Zn₅₀ treatments recorded the highest calorie values, reaching 382.80 and 380.28 calories 100 g⁻¹ of dry weight, respectively. In contrast, the Mix₁₀₀ treatment had the lowest calorie content, reaching 365.24 calories 100 g⁻¹ of dry weight. The superiority of the Mn₁₀₀ and Fe₅₀ treatments in calorie content can be attributed to their higher levels of carbohydrates and fats. The analysis revealed a positive correlation between calorie content and the fruit bodies' carbohydrate and fat content, with correlation coefficients of 0.322 and 0.450, respectively. It is well-established that each gram of carbohydrates or protein provides 4 calories, while each gram of fat provides 9 calories. Therefore, mushroom fruit bodies raised from treatments with higher levels of fat and carbohydrates would naturally have higher calorie content. These findings align with previous research, such as Carneiro et al. (2013), and are consistent with Jacinto et al. (2021) regarding crude fiber content and Manzi et al. (2001) regarding dry matter content.

Table (2) Effect of the microelements supplemented in the casing layer on the dry matter, crude fiber and calorie content of the edible mushroom *Agaricus bisporus* fruit bodies

Treatment	Chemical components		
	Dry matter (%)	Crude fiber (%)	Calories (100 g ⁻¹ of dry weight)
Fe₅₀	8.61 a-c	12.20 gf	380.28 ab
Fe₁₀₀	7.25 d	13.00 g	377.30 b-d
Fe₂₀₀	8.18 a-d	15.40 ef	373.35 e
Mn₅₀	7.91 b-d	17.20 bcd	379.33 ab
Mn₁₀₀	8.02 b-d	17.50 bc	382.80 a
Mn₂₀₀	8.97 ab	18.30 b	373.51 de
Zn₅₀	7.54 cd	16.92 cd	379.92 ab
Zn₁₀₀	9.06 ab	19.80 a	377.11 cde
Zn₂₀₀	8.62 a-c	14.90 ef	378.52 bc
Mo₅₀	7.16 d	14.52 f	377.73 bc
Mo₁₀₀	8.57 a-c	13.30 g	368.33 fg
Mo₂₀₀	9.11 ab	12.20 gh	369.32 f
Mix₅₀	7.95 b-d	15.00 ef	375.24 c-e
Mix₁₀₀	9.39 a	18.40 b	365.24 g
Mix₂₀₀	7.98 b-d	16.00 de	369.42 f
Control	7.33 cd	11.50 h	367.14 fg

*Similar letters indicate that there are no significant differences according to Duncan's multiple test at the probability level 0.05.

Conclusion

In conclusion, Molybdenum at 100 mg L⁻¹ showed remarkable results, yielding the highest protein and ash contents in *Agaricus bisporus* mushrooms. Additionally, manganese at 50 mg L⁻¹ exhibited superior total carbohydrate content. The microelements mixture at 100 mg L⁻¹ notably increased dry matter content. Furthermore, manganese at 100 mg L⁻¹ and molybdenum at 100 mg L⁻¹ outperformed other treatments in terms of fat content. Zinc at 100 mg L⁻¹ demonstrated a substantial increase in raw fiber content, while manganese at 100 mg L⁻¹ recorded the highest caloric value. These findings highlight the potential for optimizing microelement concentrations to enhance the nutritional composition of *Agaricus bisporus* mushrooms.

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