

Theses of the Doctoral (PhD) Dissertation

Development and Evaluation of Late Oyster Mushroom (*Pleurotus ostreatus* L.)-Based Feed Supplements in Japanese Quail (*Coturnix japonica*) Models

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Debrecen, 2026

1. Background and Objectives of the Doctoral Dissertation

Pleurotus ostreatus (Jacq.) P. Kummer (Pleurotaceae, Agaricomycetes), commonly known as the oyster mushroom, is an edible fungus of outstanding medicinal (TÖRÖS et al., 2022) and nutritional importance (WANG, 2020), which also significantly contributes to sustainability (EL-RAMADY et al., 2022). Due to their health benefits, mushrooms are receiving increasing attention as so-called “superfoods” (LLANAJ et al., 2023; AKTER et al., 2022). In addition to their medicinal properties, they are widely applied in biotechnology and in the cost-effective biosynthesis of nanoparticles (EL-RAMADY et al., 2022; ELSAKHAWY et al., 2022).

Functional foods and feed additives derived from mushrooms offer a natural alternative to antibiotics used in animal husbandry. According to research, *P. ostreatus* exhibits considerable immunomodulatory, antioxidant, antimicrobial, and prebiotic effects, which may positively impact poultry health and productivity (SINGH – BHARDWAJ, 2023; TÖRÖS et al., 2023). Among the mushroom’s polysaccharides, β -glucans stand out by contributing to a favorable gut microbiome composition through suppressing harmful microorganisms and promoting the proliferation of beneficial bacteria (ASSEMIE – ABAYA, 2022; MIHAI et al., 2022). Phenols and flavonoids are potent antioxidant compounds that mitigate oxidative stress-related damage, a recurrent issue negatively impacting production indices in the poultry sector (GIANNENAS et al., 2010; GIANNENAS et al., 2011).

The method of processing greatly influences the quantity and bioavailability of bioactive components. Moreover, the compositional differences between fractions are also significant. During the Maillard reaction, melanoidins and carbon nanoparticles may be formed, which exhibit additional antioxidant, antimicrobial, and prebiotic potential. Due to their biocompatibility and photoluminescence, these compounds have also gained attention in nanotechnology applications (SUN et al., 2019; LYSENKO et al., 2024; TEJWAN et al., 2022). Heat treatment simultaneously facilitates the breakdown of the cell wall, promoting the release of bioactive compounds (SHAHIDI – YEO, 2016; IZHAM et al., 2022).

In the field of poultry nutrition, *P. ostreatus*-based supplements have improved growth performance, enhanced immune response, and reduced infection risks in several animal studies. These effects may contribute to lowering antibiotic usage and thus limiting the spread of resistant strains (ABOU ZEID et al., 2019; ROMERO-ARENAS et al., 2023; TÖRÖS et al., 2024; FAROOQ et al., 2022).

The role of β -glucans is vital, as they support natural immunity and maintain the physiological state of the intestinal tract (DANESHMAND et al., 2011; HASSAN et al., 2020; SOŁOWIEJ et al., 2023; SUBERU et al., 2024).

OBJECTIVES

In our research, we aimed to investigate the effects of oyster mushroom powders produced under different processing conditions. The specific objectives were as follows:

2. Feed supplement products rich in biologically active compounds are produced from mushroom fruiting bodies and mycelium through prolonged heat treatment at low temperature under controlled humidity conditions, followed by regulated drying.
3. The biological activity of oyster mushroom powders (derived from fruiting bodies and mushroom broth) is investigated, including their effects on the growth of beneficial and pathogenic microorganisms, as well as their antioxidant functional properties.
4. The effects of the preparations on selected production parameters in Japanese quail models are evaluated, including live body weight, actual spleen weight, average weekly weight gain, total body weight gain, and weekly body weight, and the results are compared with those of untreated and β -glucan-treated groups.
5. The effects of the feed supplement model materials (oyster mushroom powders) on immunological parameters in Japanese quails are assessed, including the cytokine levels of interleukin-1 β (IL-1 β), interleukin-2 (IL-2), and interleukin-4 (IL-4).
6. Furthermore, the total antioxidant capacity of blood plasma samples obtained from the different treatment groups is determined in order to evaluate the efficiency of the antioxidant defense system and the level of cellular oxidative stress.
7. The effects of the model materials on nutrient absorption are also investigated. For this purpose, ileal samples are collected and analyzed for quantitative intestinal morphological parameters, including villus height, crypt depth, their ratio, and intestinal wall thickness.

2. Materials and Methods

The central objective of this study was to produce, chemically and microbiologically characterize, and functionally evaluate freeze-dried *P. ostreatus* (oyster mushroom-based) powders (OMP) using a male Japanese quail (*Coturnix japonica*) model following an *Escherichia coli* LPS challenge. The methodological framework was built upon three main pillars: (i) mushroom powder production and fractionation; (ii) laboratory analysis of bioactive compounds and biological activities; (iii) poultry model animal trial with sampling and a multi-parameter measurement program.

2.1 Production of Freeze-Dried *P. ostreatus* Mushroom Powders

Fresh oyster mushrooms were purchased (PENNY Market Ltd., Piacér brand, Hungary). After washing, fruiting bodies were manually quartered, placed on stainless steel trays, and pre-frozen at $-20\text{ }^{\circ}\text{C}$ for 4 hours (raw control samples).

Part of the fresh samples was cooked at $90\text{ }^{\circ}\text{C}$ for ~4 hours in a sealed vacuum cooker and drying oven (Venti-Line, VWR). At the end of the cycle, the cooked sample was centrifuged (Hajdu C28.4B, 1200 rpm/10 min) in a chamber lined with $100\text{ }\mu\text{m}$ dairy-grade filter paper. The filtrate (mushroom broth) was collected separately. Across three independent repetitions, the average yield was $57.19 \pm 0.03\%$, while the cooking loss was $30.83 \pm 2.81\%$ (due to steam release and evaporation during centrifugation).

Since direct freeze-drying of the viscous mushroom broth proved problematic, stabilization was achieved by adding 2% (w/v) agar-agar (VWR). The mixture was heated to $100\text{ }^{\circ}\text{C}$ for 5 minutes, poured onto stainless steel trays, and allowed to gel before freeze-drying. Three types of starting materials were lyophilized: (i) raw fruiting body (without cooking) → OMP-TF; (ii) cooked, centrifuged solid residue → OMP-CSR; (iii) stabilized cooked liquid fraction (mushroom broth gel) → OMP-CL.

All samples were pre-frozen at $-20\text{ }^{\circ}\text{C}$ for 4 hours, then freeze-dried at $-40\text{ }^{\circ}\text{C}$ for 24 hours (Bionanoferm Ltd.). The dried products were ground to a particle size of $\leq 100\text{ }\mu\text{m}$ and stored in airtight containers, protected from light, at $<25\text{ }^{\circ}\text{C}$ until analysis.

Summary of sample types and abbreviations:

- **OMP-TF:** lyophilized mushroom powder from whole fresh fruiting body (uncooked).
- **OMP-CSR:** lyophilized mushroom powder from cooked solid residue (remaining material after centrifugation).
- **OMP-CL:** lyophilized mushroom powder from cooked liquid fraction (filtered mushroom broth, stabilized with 2% agar-agar).

2.2 Chemical Analysis of Bioactive Compounds

2.2.1 Quantification of α -, β -, and Total Glucans

The Megazyme K-YBGL 02/21 yeast-based kit (Bray, Ireland) was used for specific determination of (1 $\rightarrow$ 3)(1 $\rightarrow$ 6)- β -glucans, as well as α - and total glucans, following the methodological description (MCCLEARY – DRAGA, 2016). UV-VIS spectrophotometry (Perkin Elmer Lambda) was used. Total glucans were hydrolyzed using exo-1,3- β -glucanase and β -glucosidase; α -glucans were digested with amyloglucosidase and invertase.

For sample preparation, 90 mg of ground mushroom powder was treated with 12 M H₂SO₄ (ice bath, 2 hours; intensive vortexing), followed by gradual dilution and incubation at 100 °C for 2 hours. Hydrolysates were neutralized with sodium acetate buffer (pH 4.5) and 8.0 M NaOH. Filtration was carried out through 0.45 μ m PTFE filters. The released glucose was measured at 510 nm after the addition of the GOPOD reagent. β -glucan values were calculated as the difference between total and α -glucan fractions. Results were expressed as w/w% (dry matter).

2.2.2 Total Polyphenol Content (TPC), Total Flavonoid Content (TFC), and DPPH Radical-Scavenging Capacity

TPC was measured at 765 nm (mg GAE/g) following SRIVASTAVA et al. (2024): 0.1 g powder extracted with 10 mL 80% methanol, reacted with Folin-Ciocalteu reagent (0.2 N, 2.5 mL) and 7.5% Na₂CO₃ (2.0 mL), incubated 30 min in the dark. Calibration with gallic acid (0–100 μ g/mL).

TFC was measured at 510 nm (mg CE/g) following WICKRAMASINGHE et al. (2023): 0.25 g powder extracted with water, followed by NaNO₂ 5% (75 μ L), AlCl₃·6H₂O 10% (150 μ L), and NaOH 1 M (0.5 mL). Calibration with catechin (20–100 μ g/mL).

DPPH radical-scavenging activity was assessed at 517 nm (SC%, inhibition) based on AHMAD et al. (2014): 1.0 mL sample mixed with 2.0 mL ethanolic DPPH solution (4×), incubated in darkness for 30 min.

All antioxidant assays were performed in triplicate using UV-VIS detection, and results were expressed on a dry weight basis.

2.2.3 Carbon Nanoparticles (CNP): Size, Mass, and Concentration

The presence and characteristics of carbon nanoparticles, assumed to form during heat treatment, were analyzed using HPLC-SEC (NGUYEN et al., 2024). Separation was performed on an Agilent AdvanceBio SEC column (300 Å; 4.6 × 300 mm; 2.7 µm) with isocratic elution (mobile phase ACN–water 20:80, flow rate 0.7 mL/min; injection volume 5 µL). Detection was achieved by UV/VIS and fluorescence (Shimadzu RF-20A; Ex 370 nm, Em 460 nm).

Calibration involved two peptide standards (Bio-Rad gel filtration standard: 1,350–670,000 Da; Merck peptide mix: 238.2–1,046.2 Da) and a glycine+glucose-based CNP standard (10–10,000 mg/kg, 4-point curve). Samples were filtered through 0.45 µm PTFE membranes. Data were processed using Agilent OpenLab CDS software. Final concentrations were calculated based on known standard concentrations and 100× diluted aqueous extracts.

2.3 Microbiological Evaluation of Freeze-Dried Mushroom Powders

2.3.1 Applied Microorganisms

For probiotic potential, *Lactiplantibacillus plantarum* (B.023391) and *Lacticaseibacillus casei* (B.01526) strains were used. Antimicrobial potential was tested against *Escherichia coli* (B.02357) and *Staphylococcus epidermidis* (B.02055). All strains were obtained from the MATE Reference Collection. Strains were stored at –80 °C in a dehydrated form and rehydrated before use in experiments.

2.3.2 Location and Methodology

Analyses were conducted at the Microbiology Laboratory, Faculty of Agricultural and Food Sciences, University of Debrecen, in accordance with food microbiology protocols. Methods included pour plate and spread plate techniques.

Media: MRS broth/agar for lactic acid bacteria; for enterobacteria and aerobic mesophiles. BioLab Ltd. supplied media and reagents.

2.3.3 Treatments and Concentrations

Five groups were tested for biological activity: 1% OMP-TF, 1% OMP-CSR, 1% OMP-CL (three oyster mushroom-based formulations), 0.1% PC (commercial β -glucan, *S. cerevisiae*; Medinvest Hungary), and NC (negative control without supplementation). Concentrations were chosen based on poultry experimental data (TÖRÖS et al., 2024).

2.3.4 Inoculation, Dilution, Seeding, and Incubation

Freshly rehydrated colonies were inoculated into liquid MRS medium and incubated at 37 °C for 18–24 h. Before assays, culture density ($OD \approx 8$) was confirmed. Test materials and sterile broth were inoculated with the starter cultures, homogenized (60 s vortex), and incubated at 37 °C for 6 h.

Serial dilutions were prepared in sterile peptone water (1.0 g peptone + 8.5 g NaCl / 1000 mL) up to 10^{-8} . *L. plantarum*, *L. casei*, and *S. epidermidis* were spread-plated (0.1 mL of 10^{-6} – 10^{-8} dilutions, triplicates), while *E. coli* was pour-plated (1.0 mL, triplicate). Incubation lasted 48 ± 2 h at 37 °C. Colonies were counted and results expressed as CFU/g on a $\lg_{10}$ scale.

2.4 *P. ostreatus* Mycelium Colonization Assay

2.4.1 Media and Sterilization

Solid media with varying mushroom broth content (0–100%) were prepared using agar-agar and distilled water, with malt extract agar (MEA) as a control. Media were sterilized by autoclaving at 121 °C for 30 min at 1–1.5 bar. After solidification in 80 mm sterile Petri dishes, media were inoculable.

Sterile stainless steel cork borers were used to cut 19 mm agar discs from pure culture plates, which were then placed in the center of the test media. Samples were incubated at 20–25 °C, protected from light, at room temperature. The observation period lasted 6 days.

2.4.2 Comprehensive Evaluation of Mycelium

Daily mycelial growth was recorded as colony diameter in millimeters. Mycelial density was visually assessed according to the 1+–5+ categories of MAHADEVAN – SHANMUGASUNDARAM (2018). After complete colonization, the mycelium was scraped off using a cell scraper, and the wet weight was measured with an analytical balance. Growth rate was calculated using the formula: (final colony diameter [mm]) / (number of elapsed days). Moisture content of the mycelium was determined by drying at 105 °C to constant weight (Venti-Line), with checks every 30 minutes. The β -glucan content of the dried mycelium was measured using the method described in Section 2.2.1.

2.5 Animal Trials on Japanese Quail Models

2.5.1 Ethical Approval, Animals, and Housing

The animal experiments were approved by the Animal Ethics Committee of the University of Debrecen (approval number: 5/2021/DEMAB) and were conducted in accordance with institutional and national guidelines. The experimental model organism was the male Japanese quail (*Coturnix japonica*), a species characterized by small body size, rapid growth, and high experimental standardization potential (NAIN et al., 2011; PUROHIT et al., 2016). Fertilized eggs were provided by Budai Quail Farm. Incubation and rearing were performed in the institutional animal facility.

The experiment started with one-day-old chicks and lasted for 28 days. Six experimental groups were established. Birds were randomly allocated to cages containing 10 individuals each. The animals were housed in cages with a stocking density of 38.85 cm² per bird under controlled environmental conditions. The initial temperature was 35 ± 3 °C, which was reduced weekly by 3 °C. Relative humidity was maintained at 60–75%. The lighting program initially consisted of 23–24 h of light, which was gradually reduced (30–40 lux), followed by a lower intensity of 10–15 lux. In addition, UVB supplementation was applied during weeks 3–4 with a photoperiod of 16–18 h.

2.5.2 Feeding Treatments and LPS Injection

The experimental groups were as follows:

- NC+SI: basal diet + physiological saline injection
- NC+LPS: basal diet + LPS injection
- PC+LPS: basal diet + 0.1% commercial β -glucan (80% w/w β -glucan, *Saccharomyces cerevisiae*)
- OMP-TF+LPS: basal diet + 1% OMP-TF
- OMP-CSR+LPS: basal diet + 1% OMP-CSR
- OMP-CL+LPS: basal diet + 1% OMP-FL

The composition of the basal diet and the nutrient content of the experimental feed mixtures are presented in Tables 10–11 of the dissertation and met or exceeded the recommendations of NRC (1994). Before the final day of the experiment, male quails received an intraperitoneal injection of *Escherichia coli* lipopolysaccharide (LPS) at a dose of 1.5 mg/kg body weight (prepared in sterile isotonic saline at 1.5 mg/mL). The placebo group (NC+SI) received sterile saline in order to control for injection-related stress effects.

2.5.3 Sampling and Identification

At the end of the 4-week experimental period, individuals were identified based on plumage characteristics, leg rings, and cloacal features. Animals were then euthanized by cervical dislocation performed under veterinary supervision.

Final body weight was recorded, and spleen weight was measured. During necropsy, blood plasma and ileum samples were collected. From the ileum, 1 cm sections were excised and placed in 4% paraformaldehyde and stored at room temperature until morphological analysis.

Blood samples were centrifuged at 3000 RCF for 10 min to obtain plasma. The plasma samples were transferred into labeled cryotubes and stored at -80°C until analysis of immune response parameters and total antioxidant capacity.

2.5.4 Laboratory Analyses

Spleen weight was measured immediately after euthanasia with a precision of ± 0.01 g. Relative spleen weight was calculated using the following equation (SUBARU et al., 2024):
$$\text{Relative spleen (\%)} = (\text{Spleen weight (g)} / \text{Body weight (g)}) \times 100$$

Body weight of male quails was measured weekly (days 7, 14, 21, and 28) using a digital balance (± 0.01 g). Weekly body weight gain was calculated as the difference between consecutive measurements: $\text{Weekly weight gain} = \text{Week}_i - \text{Week}_{i-1}$

Total body weight gain for the entire experimental period was calculated as the difference between body weight at day 28 and day 0.

Plasma interleukin levels (IL-1 β , IL-2, IL-4) were determined using ELISA (n = 6/group, duplicate measurements) according to the manufacturer's protocol (Wuhan Fine Biotech; kits: ECH0040, ECH0041, ECH0044). Absorbance was measured at 450 nm using a Synergy HTX microplate reader (BioTek). Cytokine concentrations were calculated based on a linear standard curve. The sensitivity of the assay ranged between 1.875 and 9.375 pg/mL, and the intra-assay coefficient of variation remained below 10%.

Formalin-fixed ileum samples were dehydrated through a graded ethanol series, cleared in xylene, and embedded in paraffin. Sections of 5 μm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H&E). Microscopic images were captured using an Olympus microscope equipped with a DP71 digital camera (Olympus, Japan) (FISCHER et al., 2008).

Quantification was performed using Olympus CellSens Entry software according to the method described by MUNYAKA et al. (2012). The following morphological parameters were evaluated: villus height, crypt depth, villus-to-crypt ratio, villus thickness, and total villus length.

Total antioxidant capacity (TAC) of plasma samples was determined using an Abnova assay kit (n = 6, duplicate measurements) at 570 nm using a Synergy HT microplate reader. Trolox equivalents (μmol) were calculated based on the standard calibration curve.

2.6 Statistical Analysis

All results presented in tables and figures are expressed as mean $\pm$ SEM. Data organization and graphical editing were performed using Microsoft Office Excel (version 16.0). Statistical analyses were carried out using IBM SPSS Statistics version 29.0 (IBM Corp., Armonk, NY, USA). Significant differences in tables and figures are indicated by different letters.

Statistical tests were selected according to data distribution. For normally distributed variables (e.g., microbiological analyses, carbon nanoparticle content, body weight, and ileum morphological parameters including villus height, crypt depth, width, and related ratios), one-way analysis of variance (ANOVA) was applied.

For non-normally distributed variables (e.g., β -glucan, DPPH, TPC, TFC, mycelial growth and biomass yield, relative spleen weight, body weight gain, weekly weight gain, interleukin concentrations (IL-1 β , IL-2, IL-4), and total antioxidant capacity), the nonparametric Kruskal–Wallis test was used, followed by Dunn’s multiple comparison post hoc test with Bonferroni correction.

Statistical significance was set at $p < 0.05$. For parametric analyses, assumptions of normality and homogeneity of variances were evaluated where applicable. In the animal experiments, no observations were excluded as outliers; therefore, all data points were retained in order to reflect biological variability among the animals.

3. Results and their evaluation

3.1 Characterization and investigation of the biological activity of freeze-dried *P. ostreatus* mushroom powders

3.1.1 Polysaccharide fraction

As shown in *figure 1*, the OMP-CL sample had the lowest β -glucan content (20.27 ± 0.58 w/w%), while OMP-CSR (37.95 ± 0.78 w/w%) and OMP-TF (36.25 ± 0.50 w/w%) exhibited significantly higher values. According to the literature, *P. ostreatus* and other edible mushroom species also contain considerable amounts of β -glucans, which can reach up to 40 g/100 g of dry matter (MCCLEARY – DRAGA 2016; MIROŃCZUK-CHODAKOWSKA – WITKOWSKA 2020). Our data are close to these reported values, and the differences between the various fractions are clearly observable.

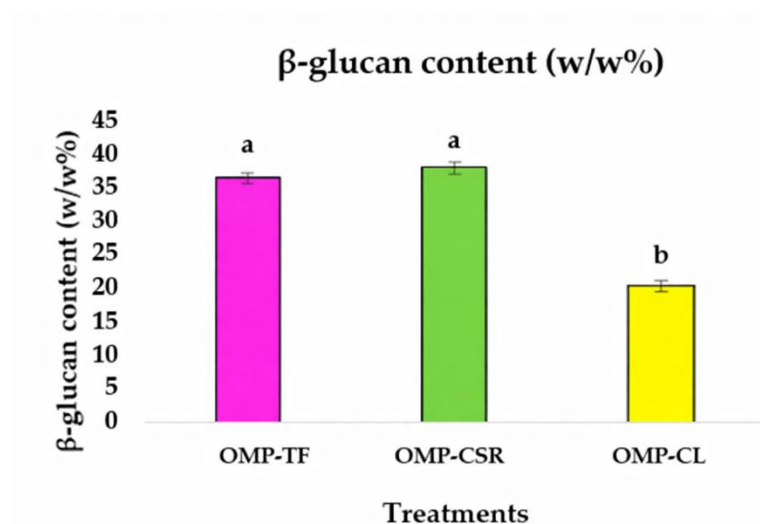


figure 1. Presentation of the β -glucan measurement results

3.1.2 Identification of carbon nanoparticles

As shown in *figure 2*, the molecular weight of carbon nanoparticles derived from different fractions was nearly identical. However, the fluorescence intensity (176.72 ± 2.00 A.u.) and concentration (50.4 ± 0.5 mg/kg) measured in the OMP-CL sample significantly exceeded the values of OMP-CSR (30.48 ± 0.46 A.u.; 8.6 ± 0.3 mg/kg) and OMP-TF (44.96 ± 7.20 A.u.; 13.1 ± 0.7 mg/kg). This indicates that the boiled mushroom broth fraction is particularly rich in water-soluble nanoparticles. Previously, carbon nanoparticles were produced from edible mushrooms using hydrothermal treatment (VENKATESWARLU et al., 2018). The novelty of our study lies in the finding that freeze-drying and milder heat treatments may also promote the formation of these nanosized materials. HUANG et al. (2019) and PARK et al. (2014) synthesized similar structures from food by-products, but they did not report nanoparticle formation under gentler processing conditions.

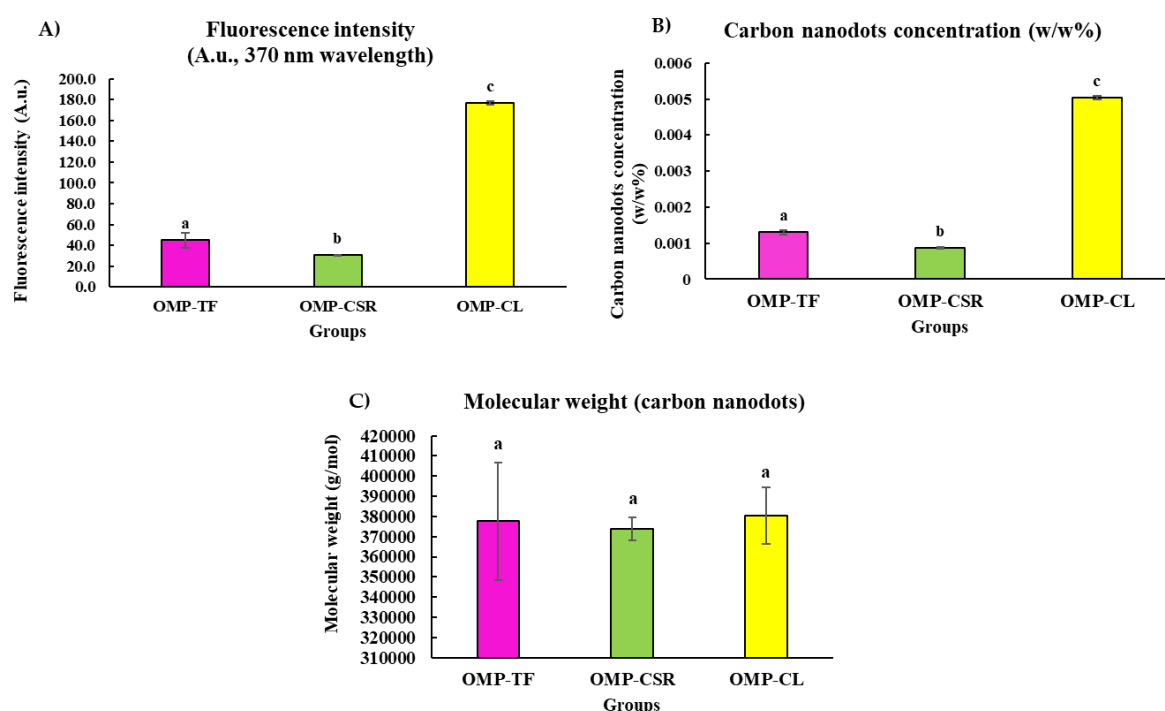


figure 2. Experimental results related to carbon nanoparticles: A) Fluorescence intensity (A.u.) at 370 nm wavelength, B) Final concentration (expressed in w/w%), C) Comparison of the molecular weight of OMPs (g/mol): (mean $\pm$ SD)

3.1.3 Antioxidant compounds and activity

As shown in *figure 3*, OMP-TF and OMP-CSR exhibited significantly higher antioxidant activity compared to OMP-CL. The total polyphenol content was 489.42 ± 97.95 mg GAE/100 g in OMP-TF and 396.23 ± 95.68 mg GAE/100 g in OMP-CSR. In contrast, OMP-CL reached only 98.86 ± 17.23 mg GAE/100 g. Similar differences were found for flavonoid content and DPPH activity: OMP-TF showed the strongest antioxidant profile, whereas OMP-CL exhibited the weakest. The results are consistent with studies highlighting that the processing of mushrooms influences their bioactive components. For example, AHMED et al. (2024) emphasized that many polyphenols and flavonoids may degrade during heat treatment. In light of my findings, the powder prepared from whole fresh mushrooms best preserves antioxidant compounds that are sensitive to heat and oxidation.

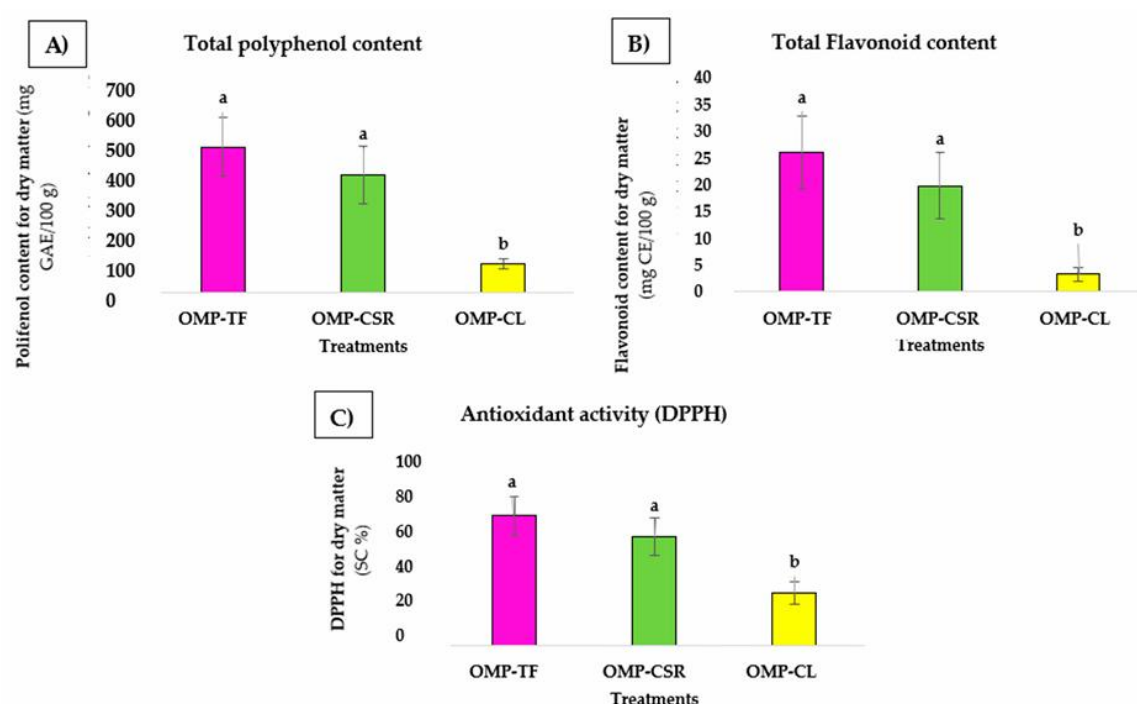


figure 3: A) Total polyphenols, B) Flavonoid content, and C) Antioxidant activity (DPPH)

3.1.4 Microbiological investigations

In the case of *L. plantarum*, no significant differences were observed between the different mushroom powders. However, *L. casei* showed a substantial increase with 1% OMP-CL (figure 4.) compared to the negative control (7.88 ± 0.16 log CFU/g). Although 1% OMP-CL had the lowest bioactive compound content, its water-soluble components and carbon nanoparticles likely served as readily accessible energy sources supporting bacterial growth. According to the literature, the prebiotic effect of mushrooms is mainly attributed to β -glucans (MIRONCZUK-CHODAKOWSKA – WITKOWSKA 2020). Additional components, such as mannitol, trehalose, or water-soluble antioxidants, may also contribute to our results. Therefore, the prebiotic effect does not depend solely on polysaccharide concentration, but also on molecular structure and the presence of accompanying compounds.

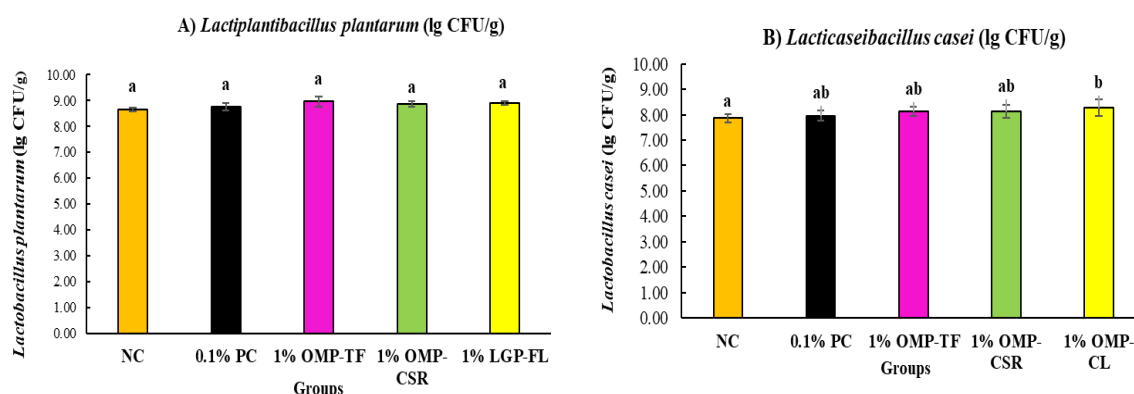


figure 4: A) Effect on the growth of *L. plantarum* and B) *L. casei*

In the case of *E. coli*, none of the samples showed a significant inhibitory effect (Figure 5). However, the growth of *S. epidermidis* was significantly reduced by OMP-CSR and OMP-CL (7.64 ± 0.19 and 7.65 ± 0.18 log CFU/g, respectively) compared to the control (8.13 ± 0.40 log CFU/g). In contrast, OMP-TF proved ineffective (figure 5.). The effect against Gram-positive bacteria is presumably due to phenolic compounds and polysaccharides released during heat treatment. My results are consistent with those of KEREZOUDI et al. (2021) and ALVES et al. (2012), who also confirmed the inhibitory ability of *P. ostreatus* against Gram-positive bacteria. According to ZHAO et al. (2022), carbon nanoparticles alone are capable of exerting antimicrobial effects; however, our study could not confirm this role.

The lack of effect against Gram-negative bacteria is likely attributable to the protective role of the lipopolysaccharide layer (LIU et al. 2024). Processing also played a significant role in shaping antibacterial activity in this case.

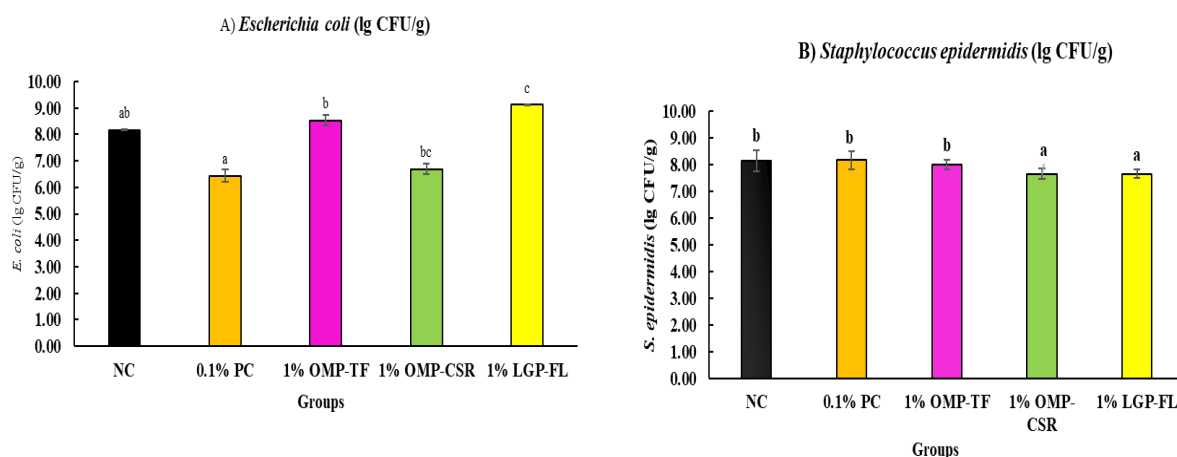


figure 5: A) Effect on the growth of *E. coli* and B) *S. epidermidis*

3.2 Optimization of mycelial β -glucan content

In the mycelium-based experiments, I aimed to investigate how different mushroom-based media affect the growth and β -glucan content of *P. ostreatus* mycelium. The main results are summarized in *table 1*. In all media, rapid mycelial growth was observed (complete colonization by day 6). While the growth rate was similar, there were significant differences in biomass yield. The highest mycelial mass was measured in the LGM-100 medium. Although biomass production was lower in the LGM-50 medium, its β -glucan content was significantly higher (11.78 ± 0.03 w/w%) compared to that in the LGM-100 medium (9.21 ± 0.05 w/w%). These results suggest that medium-concentration mushroom broth better promotes β -glucan synthesis. According to ZHANG et al. (2013), *P. ostreatus* mycelium produces varying levels of β -glucan depending on the available carbon and nitrogen sources. Similarly, CHEN et al. (2020) reported that nutrient concentration influences not only biomass but also polysaccharide biosynthesis. Optimizing medium composition is therefore critical for maximizing mycelial β -glucan yield. Considering both biomass and bioactive polysaccharide output, LGM-50 medium appears to be the most promising.

table 1: Produced biomass, moisture, and β -glucan content

Tested parameters	MEA	LGM-25	LGM-50	LGM-75	LGM-100
Produced biomass	0.23 $\pm$ 0.14a	0.47 $\pm$ 0.48ab	0.63 $\pm$ 0.66ab	0.70 $\pm$ 0.70ab	1.15 $\pm$ 1.15b
Moisture content	78.29 $\pm$ 1.49a	83.44 $\pm$ 1.46a	84.12 $\pm$ 1.45a	73.18 $\pm$ 1.98a	77.66 $\pm$ 1.28a
β -glucan content	11.04 $\pm$ 0.06ab	8.15 $\pm$ 0.04a	11.78 $\pm$ 0.03b	11.08 $\pm$ 0.01ab	11.07 $\pm$ 0.06ab
Growth rate	15	15	15	15	15
Density	4+	4+	5+	5+	5+

3.3 Results of Animal Experiments in the Japanese Quail Model

3.3.1 Body Weight, Body Weight Gain, and Weekly Body Weight Gain

As clearly illustrated in **Figure 6**, significant differences were observed in week 2 between the negative control (NC) and all mushroom-supplemented groups ($p = 0.007$): OMP-TF ($p = 0.049$), OMP-CSR ($p = 0.012$), and OMP-CL ($p = 0.036$). A similar trend was observed in week 3, where the NK group showed significantly lower body weight values compared to the mushroom-based feed supplement groups (OMP-TF: $p = 0.001$; OMP-CSR: $p < 0.001$; OMP-CL: $p < 0.001$).

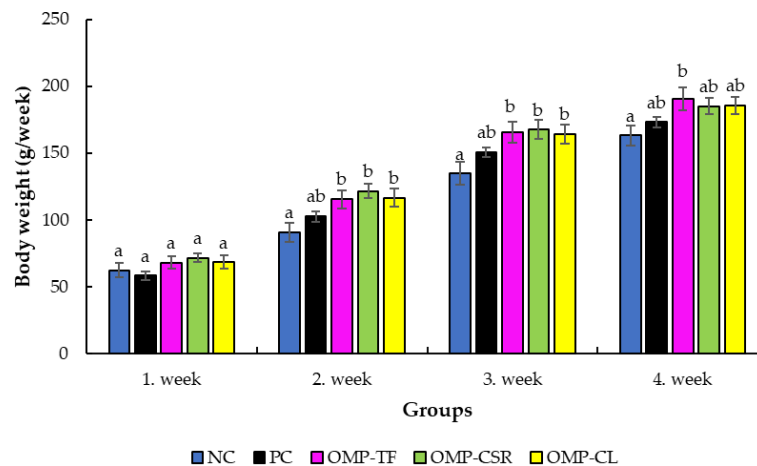


figure 6. Effect of oyster mushroom-based feed supplements on the average body weight of male Japanese quails.

When evaluating weekly body weight gain, a significant difference was observed in week 2 ($p = 0.010$), where the negative control group (NC) exhibited significantly lower weekly weight gain compared with all mushroom-supplemented groups: OMP-TF ($p = 0.005$), OMP-CSR ($p = 0.002$), and OMP-CL ($p = 0.007$), as shown in **Figure 7**.

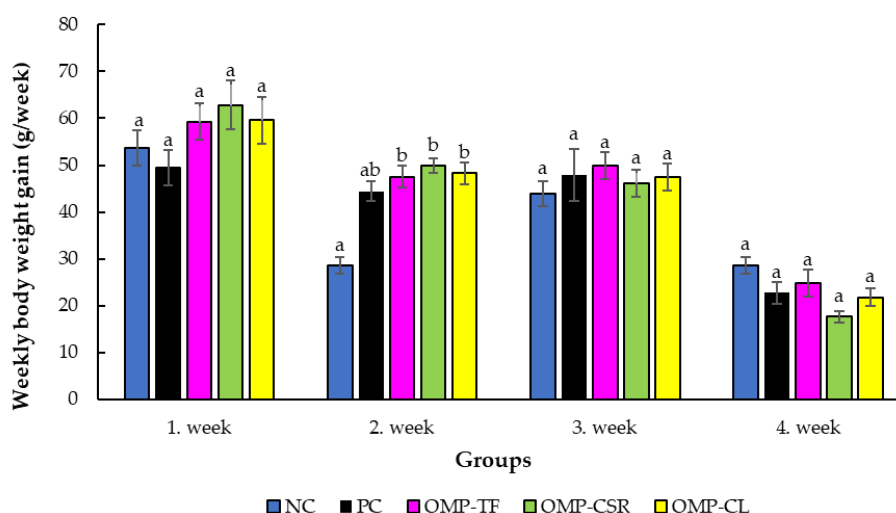


figure 7. Effect of oyster mushroom-based feed supplement products on the average weekly body weight gain of male Japanese quails.

During the experimental period, body weight gain increased significantly ($p < 0.05$) in the groups fed oyster mushroom-based feed supplements compared with the NC group: OMP-TF ($p = 0.013$), OMP-CSR ($p = 0.040$), and OMP-CL ($p = 0.014$), as illustrated in **Figure 8**.

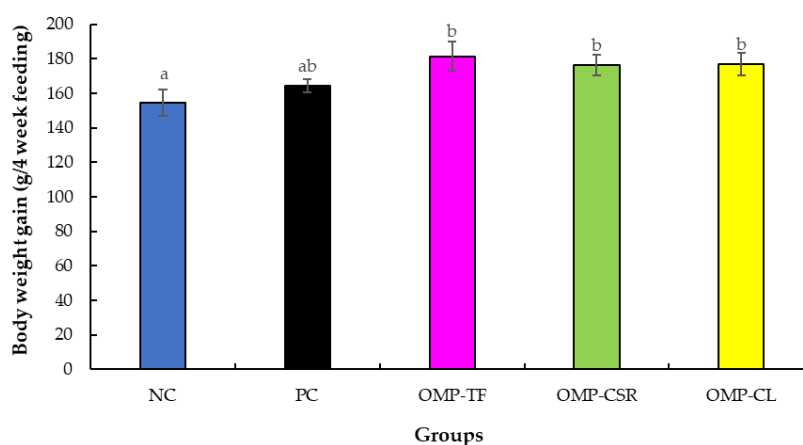


figure 8. Effect of oyster mushroom-based feed supplement products on the average body weight gain of male Japanese quails.

Several studies have demonstrated that mushroom powder-based feed supplements, including products derived from *Pleurotus ostreatus* and *Agaricus bisporus*, positively influence poultry growth performance, such as body weight gain and feed utilization (VARGAS-SÁNCHEZ et al., 2018; LIMA et al., 2021; NASIR, 2024). Our findings indicate that *P. ostreatus* supplementation improved certain growth performance parameters in male Japanese quails. The results observed on day 0 and during the first week confirm the initial homogeneity of the experimental groups, indicating that the mushroom powders did not influence early growth.

Oyster mushroom-based products increased body weight compared with the negative control during weeks 2 and 3, suggesting a potential growth-promoting effect. Although this advantage declined by week 4, the persistent effect observed in the OMP-TF group suggests that the whole-fruiting-body preparation may provide longer-term benefits. Mushroom supplementation also improved weekly body weight gain during week 2. The absence of differences during the remaining weeks may indicate that this advantage was primarily transient and most evident during early development.

3.3.2 Spleen Weight and Immune Response

The relative spleen weight (**Figure 9**) illustrates the effect of *P. ostreatus*-based supplementation on the spleen weight of male *C. japonica*. The ANOVA test did not reveal statistically significant differences among the groups ($p = 0.364$). Furthermore, the measured spleen weight range (0.06–0.13%) was very close to the normal range reported for quails of similar age (ISMAYILZADA, 2020; DUNAJEVSKA et al., 2023), indicating that the experimental samples (oyster mushroom-based feed supplements) did not induce abnormal spleen enlargement.

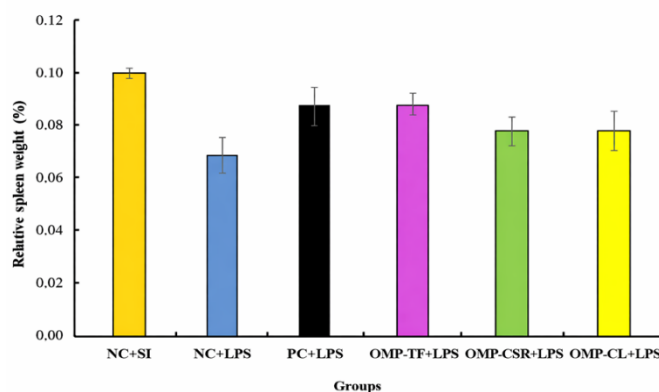


figure 9. Effect of oyster mushroom preparations and Escherichia coli LPS injection on the relative spleen weight of male Japanese quails.

The cytokine analysis results, including the concentrations of interleukins (IL-1 β , IL-2, and IL-4), are presented in **Figure 10**. None of the mushroom-supplemented LPS groups differed significantly from the LPS-treated control group (NC+LPS) in terms of IL-1 β , IL-2, or IL-4 concentrations after correction for multiple comparisons.

When examining IL-1 β concentrations, no significant differences were observed among the experimental groups ($p = 0.057$). Therefore, mushroom supplementation did not substantially modify the LPS-induced pro-inflammatory IL-1 β response under the applied experimental conditions. However, the elevated values observed in the mushroom-supplemented groups may indicate a biological response, consistent with previous studies suggesting that mushroom-derived β -glucans can stimulate the production of pro-inflammatory cytokines (COX & DALLOUL, 2010; VETVICKA et al., 2021; THOMAS et al., 2022).

In contrast, IL-2 concentrations differed significantly among the groups ($p = 0.029$); however, a significant difference was detected only between the NC+SI and OMP-CL+LPS groups ($p = 0.042$). While previous studies reported increased IL-2 production and related T-cell activation following mushroom supplementation (BENSON et al., 2019), the present results indicate that mushroom-derived products did not significantly alter the LPS-induced IL-2 response compared with the LPS-treated negative control.

Similarly, none of the mushroom-supplemented groups showed statistically significant differences in IL-4 levels compared with the LPS-treated control group (NC+LPS), suggesting that the treatments did not substantially modify the LPS-induced IL-4 response under the applied experimental conditions. According to the literature, mushroom-based feed supplements may enhance Th2-type immune responses and IL-4 production, which are important in regulating allergic and inflammatory responses (JACOB & PESCATORE, 2017). However, in the present study, no statistically significant differences in IL-4 levels were observed between the mushroom-supplemented groups and the LPS-treated control group.

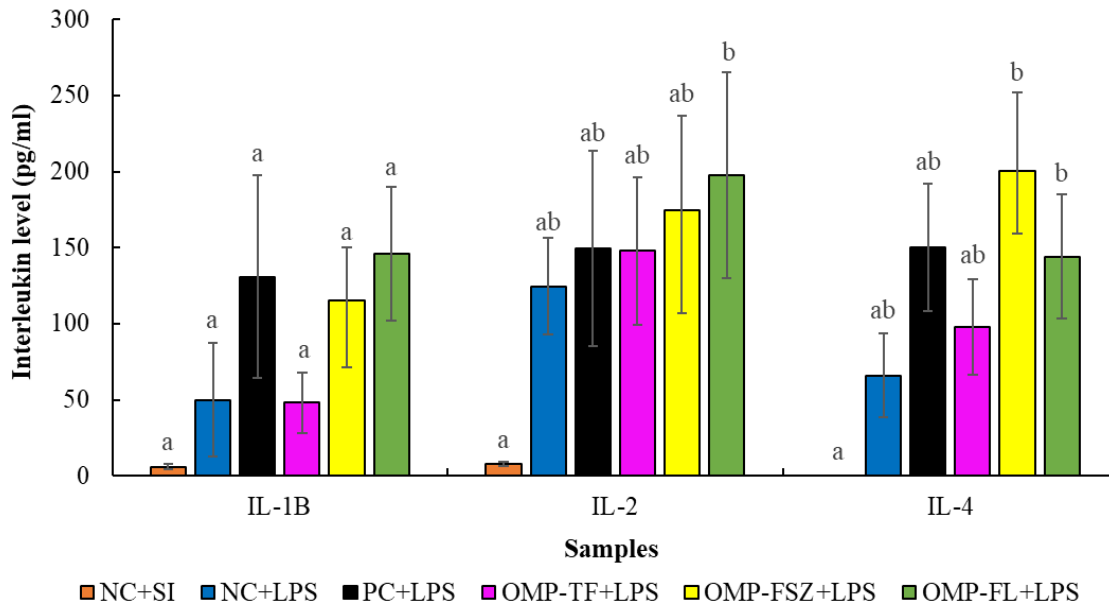


figure 10. Effect of oyster mushroom-based feed supplement products and *E. coli* LPS injection on plasma cytokine levels (IL-1 β , IL-2, IL-4) in male Japanese quails.

Evaluation of villus morphology is particularly important in the middle section (jejunum) and the distal part (ileum) of the small intestine, as these sections facilitate nutrient digestion and absorption (QIU et al., 2024). The intestinal morphological parameters observed in male Japanese quails in this study can be considered physiologically normal (NAZIR, 2022). Our results indicated no significant differences in the examined quantitative parameters, as summarized in Table 2.

table 3: The effect of Oyster mushroom products and *Escherichia coli* LPS challenge on male Japanese quails' villus morphological parameters (ileum length, depth, width, and ratios in (μm)).

Group	Villus Length	Villus Depth	Villus Length-to-Crypt Depth Ratio	Total Villus	Villus Width
NC+SI	489.28 ± 56.66	171.41 ± 12.23	2.89 ± 0.21	660.70 ± 66.45	153.70 ± 9.44
NC+LPS	452.16 ± 28.02	151.77 ± 6.22	3.04 ± 0.07	603.93 ± 34.13	136.80 ± 9.96
PC+LPS	555.83 ± 34.38	175.21 ± 11.28	3.33 ± 0.27	731.04 ± 38.01	149.87 ± 6.91
OMP-TF+LPS	492.74 ± 45.85	151.63 ± 5.07	3.35 ± 0.28	644.36 ± 47.91	137.05 ± 6.49
OMP-CSR+LPS	530.58 ± 44.51	173.38 ± 13.91	3.19 ± 0.25	703.96 ± 54.30	148.76 ± 6.90
OMP-CL+LPS	503.52 ± 46.00	149.11 ± 5.68	3.47 ± 0.29	652.63 ± 48.44	134.69 ± 6.58
p-value	0.679	0.161	0.650	0.546	0.347

The results are expressed as mean (MEAN) ± standard error of the mean (SEM) for the morphological parameters of the intestinal villi (ileum length, depth, width, and ratios in μm), based on samples collected after a 4-week (28-day) feeding period (n = 8). The obtained results did not show significant differences (p < 0.05).

3.3.3 Total Antioxidant Capacity in Blood Plasma

Figure 11 presents the effect of mushroom supplementation on total antioxidant capacity (TAC). The results are expressed as Trolox equivalents ($\mu\text{M TE}$). According to the Kruskal–Wallis test, no significant differences were observed among the experimental groups ($p = 0.281$). Total antioxidant capacity measured in poultry blood plasma provides important information about systemic antioxidant defense and the ability to counteract oxidative stress (HASSAN et al., 2020). In the present study, TAC values did not differ significantly among the experimental groups, and mushroom-based dietary supplementation did not significantly alter antioxidant capacity compared with the LPS-treated control group (NC+LPS). These results suggest that, under the applied experimental conditions, the antioxidant response was not substantially influenced by mushroom supplementation.

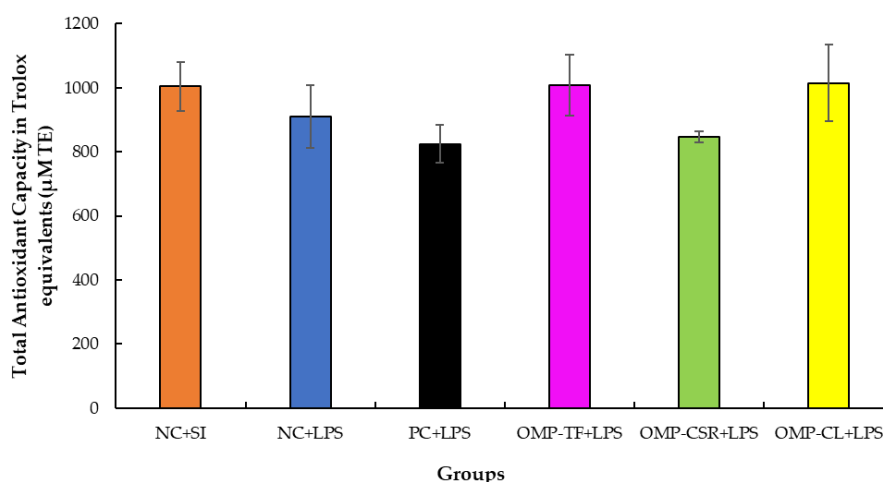


figure 11. Effect of oyster mushroom-based feed supplement products and *E. coli* LPS injection on the total antioxidant capacity (TAC) of male Japanese quails.

4. New Scientific Results of the Dissertation

During the characterization and investigation of the biological effects of different fractions of freeze-dried *P. ostreatus* mushroom powders (OMP-TF, OMP-CSR, OMP-CL), the following new scientific findings were obtained:

1. It was established that the β -glucan content of the OMP-CL mushroom powder (20.27 ± 0.58 w/w%) was substantially lower than that of OMP-CSR (37.95 ± 0.78 w/w%) and OMP-TF (36.25 ± 0.50 w/w%). However, OMP-CL proved to be the most promising mushroom-derived carbon nanoparticle source (50.4 ± 0.5 mg/kg; fluorescence: 176.72 ± 2.00 A.U.). In microbiological assays, LGP-FL at 1% concentration promoted the growth of *Lactobacillus casei* while inhibiting *Staphylococcus epidermidis*.
2. In poultry experiments, body weight values measured at weeks 2, 3, and 4 were markedly higher in certain mushroom powder-supplemented groups compared to the negative control (NC; week 2: 90.7 g, week 3: 134.6 g, week 4: 163.1 g), namely OMP-TF (week 2: 115.5 g, week 3: 165.4 g, week 4: 190.2 g), OMP-CSR (week 2: 121.4 g, week 3: 167.5 g), and OMP-CL (week 2: 116.6 g, week 3: 164.0 g).
3. When evaluating weekly body weight gain in Japanese quails, a significant difference was observed at week 2 compared to the negative control (28.5 g/week), favoring the mushroom powder-supplemented groups: OMP-TF (47.5 g/week), OMP-CSR (49.8 g/week), and OMP-CL (48.2 g/week).
4. Overall, all mushroom powder treatments (OMP-TF: 181.5 g/4-week feeding period; OMP-CSR: 176.4 g/4-week feeding period; OMP-CL: 177.0 g/4-week feeding period) enhanced body weight gain in the animal models compared to the negative control (NC: 154.6 g/4-week feeding period).
5. Following LPS injection, mushroom powder supplementation did not alter the relative spleen weight, which remained within a normal range (0.06–0.13%). Furthermore, plasma levels of IL-1 β , IL-2, and IL-4 did not differ significantly compared to the NC+LPS group (IL-1 β : 49.91 ± 37.32 pg/mL; IL-2: 124.50 ± 31.80 pg/mL; IL-4: 65.89 ± 27.68 pg/mL).
6. No statistically significant differences were observed in total antioxidant capacity (TAC) compared to the NC+LPS group (909 ± 98 μ M TE): OMP-TF (1009 ± 95 μ M TE), OMP-CSR (847 ± 17 μ M TE), and OMP-CL (1014 ± 119 μ M TE).

7. A mushroom-based culture medium (LGA) was developed, which significantly enhanced *Pleurotus ostreatus* mycelial biomass production: the value measured on LGA-100 medium (1.15 ± 1.15 g) was significantly higher than that obtained on conventional MEA medium (0.23 ± 0.14 g).

5. Practical Applicability of the Results

Based on the composition and biological activities of the freeze-dried *P. ostreatus* mushroom powders (OMP-TF, OMP-CSR, OMP-CL) examined in this research, several practical applications were identified:

1. The OMP-CSR fraction proved to be effective in terms of β -glucan content; however, no positive effect could be confirmed regarding IL-1 β , IL-2, and IL-4 levels. Due to their β -glucan content, the application of oyster mushroom powders in different fractions may potentially contribute to the modulation of immune responses in the poultry sector; however, further investigations are required to confirm this assumption.
2. My results demonstrated that the OMP-CL fraction, due to its significant fluorescence intensity and high carbon nanoparticle content, may represent a promising basis for further biotechnological research and applications.
3. In my experiments, OMP-TF, OMP-CSR, and OMP-CL oyster mushroom powder-based feed supplements significantly increased the body weight of Japanese quails during weeks 2–3, the weekly body weight gain in week 2, and the total body weight gain over the 4-week feeding period compared with the negative control (NC). Based on these findings, oyster mushroom powder-based feed supplements may potentially be applied as natural growth-promoting additives in poultry production. However, future studies should include additional poultry models as well as measurements of feed intake.
4. A novel oyster mushroom-based culture medium (LGA-50, LGA-75, LGA-100) was developed, which significantly increased mycelial biomass yield compared with the conventional MEA medium while maintaining an adequate β -glucan level. This method may provide a cost-effective alternative for mycelium production. Furthermore, the resulting product may serve as a starting material for the development of mycelium-based feed supplement products.

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Registry number:
Subject:

DEENK/508/2025.PL
PhD Publication List

Candidate: Gréta Seresné Törös
Doctoral School: Doctoral School of Animal Husbandry
MTMT ID: 10082881

List of publications related to the dissertation

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1. **Seresné Törös, G.**, Prokisch, J., Peles, F.: Hőkezelési eljárások hatása a gomba minőségére.
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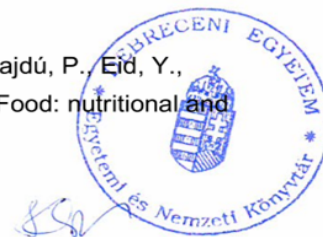
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Total IF of journals (all publications): 106,4

Total IF of journals (publications related to the dissertation): 33,4

The Candidate's publication data submitted to the Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

03 September, 2025

