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*Research Article*

# Effect of mushroom (*Pleurotus djamor* var. *roseus*) meal as feed supplemented on the hematological responses and growth performance of Nile tilapia (*Oreochromis niloticus*) fingerlings

**Luis F. Cruz-García[1], Jesús T. Ponce-Palafox[†2], Luis H. Hernández-Hernández[3], Isaac Tello-Salgado[4], Daniel Hernández-Ocampo[1] & Mario A. Benítez-Mandujano[5]**

[1]Maestría en Manejo de Recursos Naturales, Centro de Investigaciones Biológicas
Laboratorio de Bioingeniería Acuícola, Universidad Autónoma del Estado de Morelos, México
[2]Laboratorio de Calidad del Agua y Acuacultura Experimental, Universidad de Guadalajara
Puerto Vallarta, Jalisco, México
[3]Laboratorio de Producción Acuícola FES, Iztacala
Universidad Nacional Autónoma de México, México
[4]Laboratorio de Micología, Centro de Investigaciones Biológicas
Universidad Autónoma del Estado de Morelos, México
[5]Universidad Juárez de Tabasco, Unidad de los Ríos, Tenosique, Tabasco, México
Corresponding author: Isaac Tello-Salgado (hm_teonanacatl@yahoo.com.mx)

**ABSTRACT.** This study aimed to evaluate the effects of mushroom meal supplementation, *Pleurotus djamor* var. *roseus* (Pd), on the diet of Nile tilapia (*Oreochromis niloticus*), on hematological parameters and growth performance for 60 days. Nile tilapia fingerlings (3.23 ± 0.19 g) were fed with three experimental diets based on the mushroom meal in different percentages: diet 0% control (MM0), diet 15% (MM15), diet 20% (MM20) and diet 25% (MM25). Blood samples from the fish of all treatments were collected at the start of the experiment (day 0), 30 and 60 days. The weight and size of the fish were determined every 15 days (0, 15, 30, 45 and 60 days). The results showed a significant dose-dependent increase in the levels of erythrocytes count and leukocytes count. The groups that showed significant differences in the basal value and the final control result were hematocrit, hemoglobin, and lymphocyte. An increase in lymphocytes proportion and a decrease in neutrophils were found, which was related to the immune response of fish fed the diet. The highest levels of leukocytes, lymphocytes, and low neutrophils were presented in the diet with 15 and 20% ($P < 0.05$). The growth (weight gain and specific growth rate) of fish fed MM15 and MM20 of Pd in the diet was higher than the control group ($P > 0.05$). Dietary supplementation (15 and 20%) improved hematological and defense blood cells, as well as the growth of tilapia.

**Keywords:** *Oreochromis niloticus*; mushroom meal; blood cells; white cells; hematocrit; specific growth rate

## INTRODUCTION

Aquaculture tends to optimize processes to be more profitable, and intensive management of organisms can cause disease outbreaks with economic losses (Abdel-Latif et al. 2020). In tilapia (*Oreochromis* spp.) culture, the incidence of diseases has increased with its intensification (Ali et al. 2020). Therefore, the diagnosis of diseases and recently some factors that improve the immunity of tilapia have been studied (Elgendy et al. 2016, Abdel-Razek et al. 2019, Neamat-Allah et al. 2019). However, several strategies have been employed to prevent and control diseases, such as vaccines, antibiotics, and chemotherapeutics (Ramesh & Souissi 2018). However, new natural alternatives have a better acceptance by using immunostimulants as dietary additives to maintain the health of fish and improve their growth, which can prevent and control pathogens in aquaculture (Heuer et al. 2009). It is important to consider the quality of food, the physiological well-being of fish, and the effects on the environment, so functional foods are one of the most practical and pro-

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mising tools in aquaculture (Makled et al. 2017). Among natural products with high potential are mushrooms, a viable alternative in preparing foods that can provide high nutritional value and immunostimulant properties for fish's good development and growth within the culture (Ahmed et al. 2017). Among the groups with the greatest potential is the mushroom *Pleurotus* spp., the most cultivated globally for being edible and nutritious (Singhal et al. 2019). Studies on polypeptides containing mushrooms of the genus *Pleurotus* have shown that this mushroom grouphas potential in functional foods as a natural and anti-virus agent with antioxidant and immunostimulatory activities (Sun et al. 2017). Mushroom (*Pleurotus ostreatus*) can improve fish growth and stress responses by promoting organisms' better physiological and metabolic conditions in culture conditions (Khalafalla & El-Sayed 2015). The response of Nile tilapia (*Oreochromis niloticus*), carp (*Cyprinus carpio*), and rainbow trout (*Oncorhynchus mykiss*) to the inclusion of mushrooms in the diet, mainly of the *Cordyceps* and *Pleurotus* genus, has been studied (Bilen et al. 2011, Baba et al. 2015, Ulukoy et al. 2016, Van Doan et al. 2017, Safari & Sarkheil 2018). In general, the inclusion of mushroomsin the diet has increased the hematology and physiological response, high growth rate and has functioned as immunostimulant (Hleap-Zapata et al. 2021). However, the study of the effect of *Pleurotus* mushrooms on the blood response and serum biochemical parameters in tilapia has been limited. This study aimed to evaluate the effects of supplementation in 0, 15, 20, and 25% with the mushroom meal, *Pleurotus djamor* var. *roseus* in the diet of Nile tilapia fingerlings on the hematological parameters and growth performance.

## MATERIALS AND METHODS

### Preparation of mushroom meal and experimental diet

The mushroom *Pleurotus djamor* var. *roseus* (Pd) was obtained from the mushroom production module of the Mycology Laboratory of the Biological Research Center of the Autonomous University of the State of Morelos, Mexico. A sample of 23.4 kg of fresh mushroom was obtained from this module. The sample was dried in an oven at 60°C for 48 h and subsequently ground to obtain mushroom meal (MM) containing 221 g $kg^{-1}$ protein; 11.2 g $kg^{-1}$ lipids; 25.3 g $kg^{-1}$ fiber, and 74.0 g $kg^{-1}$ ash (Fig. 1b). This before adding a mushroom meal to diets. A basal diet (MM0) as a control diet was developed for this study (Table 1). For the experimental diets, Pd was used at the levels of 15, 20, and 25% inclusion in the diet. Water was added to the diet ingredients to form a uniform paste, and then pellets were made by passing them through a 4 mm diameter meat grinder. Finally, the diets were dried at 30°C for 24 h and stored at 4°C until use. Mushroom meals and diets were determined for their proximal composition of moisture, crude protein, lipids, fiber, ash content, and gross energy, according to AOAC (1990).


**Figure 1.** Characteristic of mushroom *Pleurotus djamor* var. *roseus*. a) Basidiomata *P. djamor* grown barley straw, b) mushroom meal.

### Animal and experimental design

Tilapia fingerling *Oreochromis niloticus* (3.23 ± 0.19 g) were supplied from the "Rubio" Aquaculture Production Unit, Zacatepec, Morelos, Mexico. The fish were randomly distributed in 12 glass tanks (40 L, each), with a density of 10 fish $tank^{-1}$, fed on experimental diets for 60 days (Table 1).The fish were fed three times a day (08:00, 13:00 and 18:00 h), with experimental diets at 7% of their biomass per treatment; they were kept under a photoperiod of 12:12 h (light: darkness). The recirculation system had a daily water exchange of 20% to maintain water quality in each tank. The water quality variables that were measured once a day (10:00 h) were the water temperature and dissolved oxygen with a multiprobe (YSI Model 55, Yellow Springs, OH 45387 USA), and pH (Hanna, HI98127) were monitored daily and kept at 27.0 ± 1.0°C, 6.50 ± 0.50 mg $L^{-1}$ and 7.2 ± 0.2, respectively. The basal diet (35% protein and 8.8% lipid) was supplemented with different levels of inclusion of mushroom meal, according to the levels used for *Pleurotus* by Khalafalla & EL-Sayed (2015) and Srichanun et al. (2017) in *O. niloticus* as follows: diet 0% control (MM0), diet 15% (MM15), diet 20% (MM20) and diet 25% (MP25) (Table 1).The experimental procedures and conditions were approved by the State Committee of Aquatic Health of the State of Nayarit A.C. (CESANAY) and ensured compliance with the National Service of Health, Safety, and Agrifood Quality (SENASICA) ethical guidelines.

**Table 1.** The formulation and proximate composition of experimental diet (g $kg^{-1}$dry weight). [a]Proteínas Marinas y Agropecuarias S.A. de C.V, Guadalajara, Jalisco, México. [b]Laboratorio de Micología, Centro de Investigaciones Biológicas, Universidad Autónoma del Estado de Morelos, México. [c]Vitamin and mineral mix supplemented as follows: cholecalciferol 217,000 IU; retinyl acetate 1,085,000 IU; D, L-a-tocopherol acetate 0.5 g; pyridoxine hydrochloride 0.5 g; thiamin nitrate 0.5 g; niacin 3 g; folic 0.05 g; cyanocobalamin 10 g; Ca pantothenate 1 g $kg^{-1}$; zinc 1 g; inositol 0.5 g; copper 0.25 g; iodine 0.05 g; manganese 1.32 g; sodium 7.85 g. Vitamin C 98% 5 g. [d]Grupo Trimex, Ciudad de México, México. GE: gross energy, CMC: sodium carboxymethyl cellulose.

| Ingredient | Diets (g $kg^{-1}$ dry weight) | | | |
|---|---|---|---|---|
| | MM0 | MM15 | MM20 | MM25 |
| Fish meal[a] | 343 | 145 | 113 | 81 |
| Soybean meal | 400 | 400 | 400 | 400 |
| Mushroom meal[b] | 0 | 198 | 265 | 329 |
| Fish oil[a] | 100 | 100 | 100 | 100 |
| Premix[c] | 40 | 40 | 40 | 40 |
| Dextrinized starch[d] | 67 | 67 | 32 | 0 |
| CMC[a] | 50 | 50 | 50 | 50 |
| Proximate composition of the experimental diets (g $kg^{-1}$ dry weight basis) | | | | |
| Crude protein | 352.9 | 352.6 | 352.3 | 352.0 |
| Crude lipid | 87.8 | 87.9 | 88.1 | 88.3 |
| Moisture | | | | |
| Ash | 76.1 | 76.6 | 76.8 | 76.9 |
| Fiber | 49.3 | 50.1 | 50.5 | 50.9 |
| GE (kcal $g^{-1}$) | 4.22 | 4.21 | 4.20 | 4.19 |

**Hematological parameters**

Three fish from each tank were anesthetized using clove powder at 500 mg $L^{-1}$ (Safari & Sarkheil 2018). Blood samples were extracted from the gill area where the ventral aortic vein is located using a syringe with EDTA anticoagulant, approximately 1.5 mL of blood (Campbell & Ellis 2007) on days 1, 30, and 60 of each treatment. Samples were placed and stored in heparinized tubes tomeasure hematological parameters as erythrocytes count, hematocrit (Ht), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), leukocytes count, and differential white blood cell count (WBCs)as neutrophils, eosinophils, basophils, lymphocytes, and monocytes (Blaxhall & Daisley 1973).

Samples were taken using a pipette up to the 0.5 unit mark, complementing with the 1% Hayem solution until the 101 unit mark without bubbles was mixed for 2 or 3 min to determine red blood cell (RBC). For leukocytes, 0.5 units of blood samples were taken with a white blood cell pipette. Subsequently, the 1% Turk solution was taken up to the 11-unit mark without bubbles, mixed for 3 min. The cell count of the two parameters was carried out with the first three drops (each drop is 25 µL), and the next one was placed in the Newbauer chamber, and the cells were counted in the five squares of the central reticle. The blood cell count is expressed in cells $mm^{-3}$ (Campbell & Ellis 2007). The Ht was determined by separating plasma samples from the blood after centrifugation at 3000 g for 5 min at 4°C using a centrifuge for hematology (Model: M 19, 10,000 rpm). All components were analyzed using a clinical kit (Asan Pharm. Co., Ltd.). Hemoglobin Cal Kit was used to determine Hb, consisting of Drabkin diluent and standard solution (Campbell & Ellis 2007). Four-point nine milliliters of distilled water were taken for the diluent, and 0.1 mL of Drabkin's solution was added; this same solution was used as a blank and one more for the standard solution (reference value), the above was done with each sample of each organism (20 µL). Reading was performed in a spectrophotometer at a wavelength of 540 nm; the result is obtained once the kit makes the given formula for the Hb concentration in g $dL^{-1}$ (Blaxhall & Daisley 1973). From the results of Hb concentration, erythrocyte count and Ht, erythrocyte indices such as MCV = (Ht × 10) / total erythrocyte count, fL; MCHC = (Hb × 100) / Ht, g $dL^{-1}$; and MCH = (Hb × 10) / total erythrocyte count, pg; were obtained (Lee et al. 1998).

**Morphological samples of blood cells**

Morphological examination of the primary blood cells was carried out to show that the treatment cells' general characteristics and better growth were within the known pattern. It was done by blood smear and optical microscopy (Nikon Eclipse 50i. 12 M.G. resolution camera). Blood smears were made from fish-fed diets

that had 0-25% inclusion of mushroom meal. These were fixed in methanol for 3 min at room temperature, stained with Giemsa (Solarbio, China) using the method of Chen (Chen et al. 2019). Pictures of stained smears were taken with a Nikon 50i Microphot.

### Growth performance

Fishes were measured and weighed at the beginning of the experiment and then every 15 days until the experimental period. The initial length (IL, cm), initial body weight (IBW, g), final length (FL, cm), final body weight (FBW, g) were determined. The growth performance was calculated at the end of the feeding trial, using the following formulas:

Specific growth rate (SGR%) (g $d^{-1}$) = 100 × ln final weight - ln initial weight / time of experiment,

Weight gain (WG) = final weight (g) - initial weight (g);

Feed conversion ratio (FCR) = feed given / weight gain and survival rate (%) = (final individuals number / initial individuals number) × 100 (Van Doan et al. 2017).

### Statistical analysis

The normality was confirmed with the Kolmogorov-Smirnov test and homoscedasticity with Bartlett's test before data analysis. The variables expressed in percentages were transformed with arcsin $\sqrt{x} + 1$ for their analysis. One-way analysis of variance (ANOVA) followed by the Tukey test was used to compare the baseline values with the final values. The mean values were considered significantly different when $P < 0.05$. All statistical analysis was performed by Statistica 10.0 Stat Soft, Inc. 1984-2011Computer Program.

## RESULTS

### Hematological parameters

The variations of the main hematological parameters of the Nile tilapia *Oreochromis niloticus* fed with 0, 15, 20, and 25% inclusion of mushroom Pd in the diet at 0, 30, and 60 days are shown (Figs. 2-3). A significant increase ($P < 0.05$) was found in the concentration of Hb, Ht, erythrocytes count, and leukocytes count and lymphocytes proportion from 30 to 60 days of the experiment. Neutrophils in the treatments with mushroom meals showed a significant decrease (Fig. 3b).

The initial and final concentrations of all the hematological parameters determined in this study are shown (Table 2). In the control treatment, the Hb, MCH, MCHC, and basophils had a significant ($P < 0.05$) higher concentration about time, at 60 days of the experiment. There was no significant difference ($P > 0.05$) in the control treatment in all other parameters. The mean values of MCV did not have significant differences ($P > 0.05$) in all treatments. Lymphocytes and neutrophils were the most common types of leukocytes 29.6-47.4 and 43.8-60.8%, respectively.

### Cell morphology in blood

The types of leukocytes and erythrocytes in healthy tilapias presented MM15 treatment differed from each other according to their morphology (Fig. 4). Erythrocytes and the types of leukocytes were identified. The erythrocytes were characterized by being oval, oval nucleus and, in some cases, round, and cell pleomorphism was shown (different cell forms) (Fig. 4a). Within leucocytes, the neutrophils showed an eccentric nucleus, and the reaction of their granules was neutral, so a white or light blue color was observed (Fig. 4a). Monocytes have granules, they are large, and their nucleus could be seen in several forms. The cytoplasm was observed to be light blue and sometimes darker (Fig. 4b). Thrombocytes are the cells responsible for coagulation; they are rounded with a generally eccentric globose nucleus (Fig. 4c), with basic reaction and dense chromatin. The cytoplasm is scarce in most of them, and hyaline, with discrete basophile on the periphery, is usually found agglomerated or in groups. Lymphocytes were small cells, have a nucleus that occupies almost the entire cytoplasm, and has no granules (Fig. 4d). Basophils have an eccentric nucleus, and the reaction of their granules is basic, so it shows blue in their granules (Fig. 4e). Eosinophils were round, have an eccentric nucleus, and the reaction of cytoplasmic granules is acidic, and they were orange (Fig. 4f).

### Growth performance

The growth parameters FBW, WG, SGR%, and survival were significantly higher in the diets with a mushroom meal than in control (Table 3). There were no significant differences in the FCR of all treatments.

## DISCUSSION

In recent years, hematological parameters have been used to evaluate the response to applying additives and products of natural origin and determine tilapia's health status (Reda et al. 2016). Among the innovative products of natural origin is mushroom. These organisms improve the immune system and growth (Hleap-Zapata et al. 2021). The mushroom stalk waste of *Pleurotus pulmonius* has been used in diets (23% protein) for tilapia tolerating inclusion levels of 5%

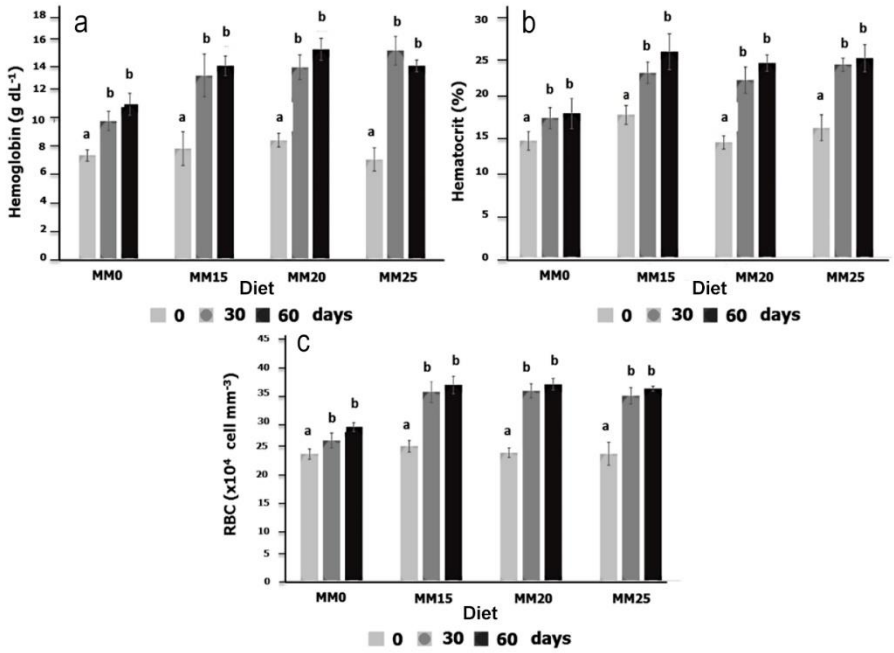


**Figure 2.** Hematological parameters of Nile tilapia *Oreochromis niloticus* during the experimental period. a) Hemoglobin, b) hematocrit, c) erythrocytes. Values with different letters within the same treatment have significant differences ($P < 0.05$) after one-way ANOVA followed by Tukey's multiple range test.

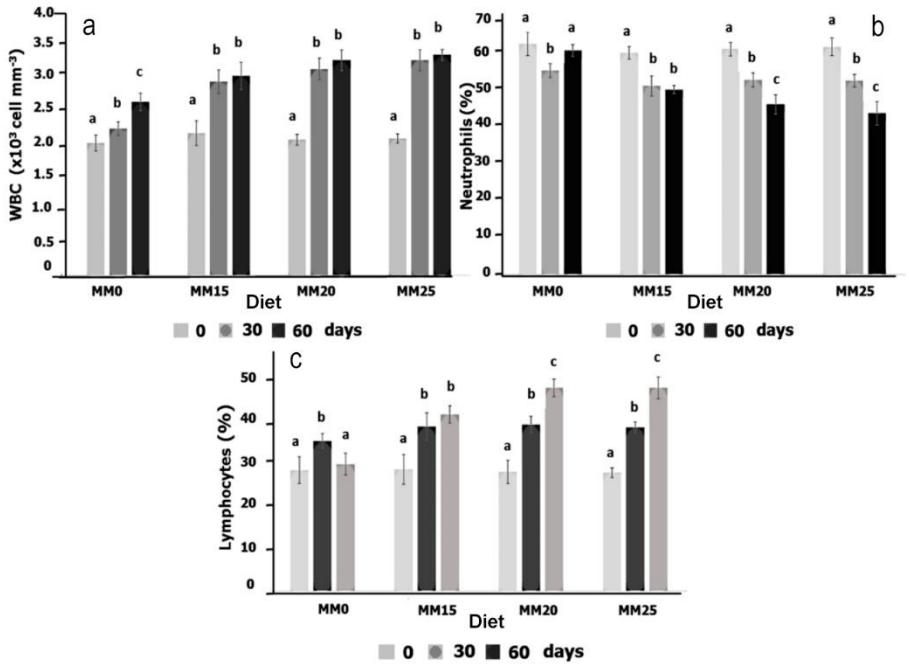


**Figure 3.** Hematological parameters of Nile tilapia *Oreochromis niloticus* during the experimental period. a) Leukocytes, b) neutrophil, c) lymphocytes. Values with different letters within the same treatment have significant differences ($P < 0.05$) after one-way ANOVA followed by Tukey's multiple range test.

maximum (Ahmed et al. 2017), a level lower than that found in our study with Pd. This study showed that the addition of 15% Pd meal in diets for tilapia improved health.

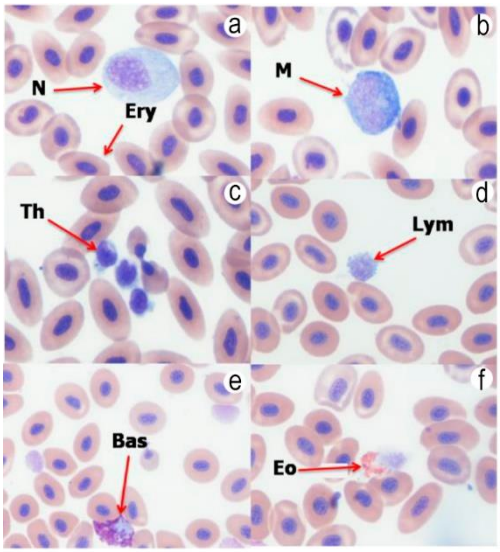


**Figure 4.** Morphology of a) N: neutrophils, Ery: erythrocytes, b) M: monocytes, c) Th: thrombocytes, d) Lym: lymphocytes, e) Bas: basophils, f) Eo: eosinophils.

The hematological parameters obtained in this study were within the ranges determined in the blood component studies for Nile tilapia *Oreochromis niloticus* (Ueda et al. 1997, Hahn von Hessberg et al. 2014). The highest percentages of leukocyte types were neutrophils, lymphocytes, monocytes, and eosinophils, which agree with other fish (Mehana et al. 2015). The decrease in the percentage of neutrophils in Nile tilapia at 60 days showed that the inclusion of Pd meal kept the levels of these cells in the normal range (0.11-63.4%) for organisms <50 g (Hahn-von-Hessberg et al. 2014) because an increase in the number of neutrophils can be a stress factor that affects the growth and welfare of tilapia (Martins et al. 2008).The morphology of the blood cells was within the typical patterns of the species (*O. niloticus*) for healthy organisms (Ueda et al. 1997, 2001).

The inclusion of Pd in the diet of Nilotic tilapia improved growth and survival parameters at the tested levels of 15 to 25% compared to the control. This difference with the control has been reported for other *Pleurotus* spp.,in the Nile tilapia feeding (Aderolu et al. 2015, Khalafalla & El-Sayed 2015 (10-40g)).However, when tested with other species like *Pleurotus florida*, there were no differences in its growth with the control (Muin et al. 2014).The WG, SGR%, and survival obtained in this study have been higher than those found when including other *Pleurotus* species in diets for Nile tilapia (Aderolu et al. 2015, Khalafalla & EL-Sayed 2015, Srichanun et al. 2017), or similar to *P. florida* (Muin et al. 2014). However, when oyster mushroom stalk waste (*Pleurotus* spp.) has been used in Nile tilapia diets, higher yields have been obtained

**Table 2.** Effects of mushroom meal (MM) on the hematological parameters of *Oreochromis niloticus* at 0 and 60 days of feeding the experimental diets. [1]Erythrocytes count, [2]leukocytes count, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration. Values with different letters within a row are significantly different (ANOVA, $P < 0.05$), and the difference is by comparison between treatments in the same sampling

| Parameter/Diet | MM0 | | MM15 | | MM20 | | MM25 | |
|---|---|---|---|---|---|---|---|---|
| Time (d) | 0 | 60 | 0 | 60 | 0 | 60 | 0 | 60 |
| Erythrocytes ($\times 10^6$ cell mm$^{-3}$)[1] | $2.4 \pm 0.1^{b}$ | $2.9 \pm 0.1^{b}$ | $2.5 \pm 0.1^{b}$ | $3.7 \pm 0.1^{a}$ | $2.4 \pm 0.1^{b}$ | $3.7 \pm 0.1^{a}$ | $2.4 \pm 0.2^{b}$ | $3.6 \pm 0.4^{a}$ |
| Leukocytes ($\times 10^5$ cell mm$^{-3}$)[2] | $2.0 \pm 0.1^{b}$ | $2.7 \pm 0.1^{b}$ | $2.2 \pm 0.2^{b}$ | $3.1 \pm 0.2^{a}$ | $2.1 \pm 0.8^{b}$ | $3.3 \pm 0.2^{a}$ | $2.1 \pm 0.1^{b}$ | $3.4 \pm 0.8^{a}$ |
| Hemoglobin (g dL$^{-1}$) | $7.5 \pm 0.4^{b}$ | $11.2 \pm 0.8^{a}$ | $8.0 \pm 1.2^{b}$ | $14.0 \pm 0.7^{a}$ | $8.6 \pm 0.5^{b}$ | $15.2 \pm 0.8^{a}$ | $7.2 \pm 0.8^{b}$ | $14.0 \pm 0.4^{a}$ |
| Hematocrit (%) | $14.4 \pm 1.1^{b}$ | $17.8 \pm 1.9^{b}$ | $17.6 \pm 1.1^{b}$ | $25.4 \pm 2.2^{a}$ | $14.2 \pm 0.8^{b}$ | $24.0 \pm 1.0^{a}$ | $16.0 \pm 1.6^{b}$ | $24.6 \pm 1.67^{a}$ |
| MCV (fl) | $60.7 \pm 4.5^{b}$ | $61.9 \pm 7.8^{b}$ | $69.9 \pm 4.7^{a}$ | $69.2 \pm 4.2^{a}$ | $58.3 \pm 4.2^{a}$ | $65.2 \pm 2.5^{a}$ | $67.6 \pm 7.9^{a}$ | $69.9 \pm 4.4^{a}$ |
| MCH (pg) | $31.5 \pm 2.7^{b}$ | $38.9 \pm 2.6^{a}$ | $31.7 \pm 4.6^{b}$ | $38.2 \pm 1.9^{a}$ | $35.3 \pm 3.2^{b}$ | $41.3 \pm 2.2^{a}$ | $30.5 \pm 5.2^{b}$ | $44.9 \pm 1.3^{a}$ |
| MCHC (g dL$^{-1}$) | $52.2 \pm 6.9^{b}$ | $63.5 \pm 8.4^{a}$ | $45.3 \pm 5.4^{b}$ | $55.4 \pm 5.4^{b}$ | $60.6 \pm 2.6^{a,b}$ | $63.5 \pm 5.4^{a}$ | $45.7 \pm 9.3^{b}$ | $64.5 \pm 5.6^{a}$ |
| Neutrophils (%) | $62.6 \pm 3.2^{b}$ | $60.8 \pm 1.6^{b}$ | $60.2 \pm 1.7^{b}$ | $50.2 \pm 1.1^{a}$ | $61.2 \pm 1.9^{b}$ | $46.2 \pm 2.6^{a}$ | $61.8 \pm 2.4^{b}$ | $43.8 \pm 3.2^{a}$ |
| Eosinophils (%) | $3.6 \pm 1.1^{a}$ | $3.0 \pm 1.6^{a}$ | $4.0 \pm 1.6^{b}$ | $3.2 \pm 0.8^{a}$ | $4.4 \pm 1.5^{b}$ | $2.2 \pm 0.8^{a}$ | $4.7 \pm 1.6^{b}$ | $4.0 \pm 1.0^{b}$ |
| Basophils (%) | $1.8 \pm 0.8^{a}$ | $2.8 \pm 0.8^{b}$ | $3.2 \pm 1.1^{b}$ | $3.0 \pm 1.2^{b}$ | $2.4 \pm 0.5^{a}$ | $1.8 \pm 0.8^{a}$ | $2.4 \pm 0.5^{a}$ | $2.2 \pm 0.8^{a}$ |
| Monocytes (%) | $3.8 \pm 0.8^{b}$ | $3.8 \pm 1.2^{b}$ | $4.2 \pm 0.8^{b}$ | $2.4 \pm 0.5^{a}$ | $4.2 \pm 0.8^{b}$ | $2.4 \pm 0.9^{a}$ | $3.2 \pm 0.8^{b}$ | $2.6 \pm 1.5^{a}$ |
| Lymphocytes (%) | $28.2 \pm 3.1^{b}$ | $29.6 \pm 2.5^{b}$ | $28.4 \pm 3.5^{b}$ | $41.2 \pm 2.0^{a}$ | $27.8 \pm 2.7^{b}$ | $47.4 \pm 2.1^{a}$ | $27.6 \pm 1.1^{b}$ | $47.4 \pm 2.5^{a}$ |

**Table 3.** Growth parameters of *Oreochromis niloticus* after 60 days of feeding the experimental diets. MM: Mushroom meal, IL: initial length fish$^{-1}$, IBW: initial body weight fish$^{-1}$, FL: final length fish$^{-1}$, FBW: final body weight fish$^{-1}$, WG: weight gain fish$^{-1}$, SGR%: specific growth rate fish$^{-1}$, FCR: feed conversion ratio. Values with different letters within a row are significantly different (ANOVA, $P < 0.05$), and the difference is by comparison between treatments in the same sampling time.

| Parameter/diet | MM0 | MM15 | MM20 | MM25 |
|---|---|---|---|---|
| IL (cm) | $4.1 \pm 0.74^a$ | $4.2 \pm 0.82^a$ | $4.2 \pm 0.26^a$ | $4.1 \pm 0.16^a$ |
| IBW (g) | $3.2 \pm 0.16^a$ | $3.2 \pm 0.22^a$ | $3.3 \pm 0.22^a$ | $3.2 \pm 0.17^a$ |
| FL (cm) | $18.3 \pm 0.57^a$ | $19.1 \pm 0.62^a$ | $18.6 \pm 0.48^a$ | $18.6 \pm 0.16^a$ |
| FBW (g) | $21.6 \pm 0.95^b$ | $30.1 \pm 1.19^a$ | $29.1 \pm 1.21^a$ | $27.1 \pm 1.10^a$ |
| WG (g) | $18.4 \pm 0.3^b$ | $26.9 \pm 0.4^a$ | $25.8 \pm 0.1^a$ | $23.9 \pm 0.6^b$ |
| SGR% (g d$^{-1}$) | $3.1 \pm 0.1^b$ | $3.7 \pm 0.2^a$ | $3.6 \pm 0.3^a$ | $3.6 \pm 0.1^a$ |
| FCR (g) | $1.4 \pm 0.2^a$ | $1.2 \pm 0.1^a$ | $1.3 \pm 0.3^a$ | $1.4 \pm 0.2^a$ |
| Survival (%) | $76.0 \pm 2.3^b$ | $94.0 \pm 3.5^a$ | $94.0 \pm 3.3^a$ | $90.0 \pm 4.5^a$ |

than those found in our study (Ahmed et al. 2017).The previous studies reported with *O. niloticus* were carried out in the ranges of weight studied (3-40 g) and time of the bioassay (60-70 days). The best response to the growth parameters in tilapia, compared to the control, could be because Pd has antioxidant, anti-inflammatory, and antitumor substances (Serrano & Divina 2016).

It has been determined that mushrooms of the genus *Pleurotus* spp. produce β-glucan that at high levels can produce toxicity (Qinghui et al. 2007). Assays of mushroom stalk waste of *P. pulmonarius* in Nile tilapia have shown that levels up to 20% β-glucan do not induce mortality in feeding trials, and the content of β-glucan in our diets did not influence survival. This study demonstrated that *Pleurotus djamor* var. *roseus* mushroom binds to several other species (*P. sajor-caju*, *P. ostreatus*, *P. albidus* and *P. flabellatus*) when used in low concentration diets as a nutritional supplement for fish (Sartori et al. 2015). *P. djamor* increases the nonspecific immune response in Nile tilapia when using feed supplemented with oyster mushroom extract. Also, it was found that the supplementation of a mushroom meal in a proportion of 15 to 25% in the diet of the fish can improve the health status, growth, and survival of Nile tilapia, which is in accordance with what has been reported for the response of tilapia to the inclusion in the diet of other species of *Pleurotus* spp. (Srichanun et al. 2017, Safari & Sarkheil 2018). This positive response of Nile tilapia to the inclusion of Pd at low levels could be because the consumption of *Pleurotus* has shown immune-stimulating activity in fish (Abdullah et al. 2017).

In conclusion, leucocyte percentage increased with mushroom meals, and neutrophils and monocytes decreased significantly ($P < 0.05$). In addition, there was a trend towards a high final weight, WG, SGR%, and survival in the diets with up to 25% inclusion of *P. djamor* meal. Even in the groups that presented alterations in the proportions of defense blood cells, there was no damage to growth rates, which can be considered positive. In a future trial, it would be interesting to test the effect of these treatments followed by an experimental infection.

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