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EFFECT OF *Lemna gibba* AND *Ulva lactuca* ON HEALTH AND PRODUCTIVE PERFORMANCE IN JUVENILES OF NILE TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

Currently, mannan-oligosaccharides (MOS) are added to the production costs of Nile tilapia to prevent and avoid diseases. An alternative are aquatic plants rich in biologically active compounds such as *Lemna gibba*, a freshwater macrophyte, and *Ulva lactuca*, a green seaweed, which have shown productive and economic efficiency as feed for livestock, poultry, and fish, but their effect as a functional ingredient has not been evaluated. The objective was to demonstrate that dietary supplementation of juvenile Nile tilapia (*Oreochromis niloticus*) with *L. gibba* and *U. lactuca* can have a beneficial effect on the health and productive performance of tilapia allowing to consider these supplements as functional ingredients such as mannan-oligosaccharides (MOS). The base diet was supplemented with *L. gibba* and *U. lactuca* at percentages 0, 10, 15 and 20 % (L0, L10, L15, L20; U0, U10, U15, U20; M0, M10, M15, M20) and compared to a mannan-oligosaccharide (MOS) control. Health parameters (hematological study, lysozyme and complement C3 activities) and productive performance (survival, S; final weight, FW; protein efficiency ratio, PER, and feed conversion factor, FCF) were evaluated. Statistical difference was found ($p \leq 0.05$) among treatments and supplementation percentages. The U20 diet showed the greatest effect ($p \leq 0.05$) on health parameters and L15 showed the best results ($p \leq 0.05$) in productive performance. It is concluded that *U. lactuca* and *L. gibba* can be used as a functional ingredient at a proportion of 15 % to produce feed for Nile tilapia juveniles in order to promote health and growth.

Keywords: *Oreochromis niloticus*, functional ingredient, health.

INTRODUCTION

Nile tilapia is a strategic species for food security (FAO, 2019) that may contribute to meeting food demand, one of the consequences of population increase and climate change in developing countries. One of the limiting factors in tilapia production and cause of large economic losses is mortality associated with diseases and ineffectiveness of the current methods used (El-Sayed, 2020). It is advisable to abandon traditional practices and prohibit the use of antibiotics that generate bacterial resistance in fish

and their consumers, in addition to contaminating the water and sediment. Therefore, available, and economically feasible alternatives are required.

One of the supplements, which adds to production costs as a multifunctional supplement, is mannan-oligosaccharides (MOS) (Yuji-Sado *et al*., 2015). Aquatic plants can be an alternative to supplements because in their natural environments they synthesize biologically active compounds to tolerate adverse environmental conditions and as defense against predators. The chemical composition and number of compounds synthesized by aquatic plants vary depending on the species, genetics, habitat, environmental conditions, exposure to solar radiation, among others (Hentati *et al*., 2020).

Among them *Lemna gibba*, a small freshwater macrophyte plant that shows accelerated growth in temperate and tropical water pools, because of its nutritional content it has been recommended for animal feed (El-Shafai *et al*., 2004). Another is *Ulva lactuca*, a green seaweed that is abundant on tropical coasts in warm months (Hentati *et al*., 2020). Both plants have been studied in livestock, poultry and fish feed with satisfactory productive results and economic efficiency (Tirado-Estrada *et al*., 2018) but have not been evaluated in farmed tilapia as an ingredient or functional feed. That is, as a natural food that in addition to being nutritious contains biologically active components that provide some beneficial effect on health and reduce the risk of contracting bacterial, fungal, or viral diseases (Beltrán de Heredia, 2016).

The possibility of sustaining the use of these aquatic plants as a functional food supplement can contribute to improve production practices. Based on the mannan-oligosaccharides (MOS) have been used to improve weight gain, serum lysozyme concentration which boosts the immune system and to prevent the adhesion of pathogenic bacteria in the intestine which promotes optimal absorption of nutrients for proper growth (Yuji-Sado *et al*., 2015). It is assumed that the study may contribute to improve production practices through the natural use of aquatic plants, towards sustainability, disease prevention and improvement in the physiological condition of tilapia. Therefore, the objective of this study was to demonstrate that dietary supplementation of juvenile Nile tilapia (*Oreochromis niloticus*) with *Lemna gibba* and *Ulva lactuca* can have a beneficial effect on the health and productive performance of tilapia, allowing to consider these supplements as functional ingredients such as mannan-oligosaccharides (MOS).

MATERIALS AND METHODS

Aquatic plants, origin, and processing

Ulva lactuca was collected in the state of Veracruz-Llave at the beaches "Norte" and "Gaviotas". *Lemna gibba* was collected at "Plan de pájaros" and "Buena vista" lakes in the municipality of Loma Bonita, Oaxaca, both during July-August 2020. The aquatic plants were washed with well water under running water. Subsequently, they were put to dry in the sun with constant turning on cloth and a mesh. A sample of each aquatic plant was taken at tearing point and then dried in an oven at 45 °C for 3 hours. Finally, each of the plants were ground. *U. lactuca* with a conventional blender and *L.*

gibba with a mortar and pestle. The grinding was passed through a 0.01 μm diameter sieve to obtain flour.

Fish and experimental conditions

Nile tilapias were purchased from TECNOPEZ S.A. the aquaculture farm, located in the state of Veracruz-Llave, transported, and handled following the guide for the care and use of laboratory animals (NRC, 2011) and the international standards of the Aquatic Animal Health Code (2019) of the World Organization for Animal Health (OIE, for its acronym in French). Juveniles of Nile tilapia (9.33 g ± 0.20) were selected for the experiment.

Experimental design

The multifactorial experiment was conducted as a completely randomized block design. Four aquaria were used per treatment, each of 80 L with three replicates in a constant recirculation system. Ten fish were randomly placed in each aquarium. Water quality was daily monitored for temperature, pH and ammonium oxygen demand using a 550 YSI probe. The experiment lasted 60 days. The mathematical model used was:

$$Y_{ij} = \mu + T_i + \beta_j + \varepsilon_{ij},$$

where Y_{ij} is the response variable, μ is the overall mean, T_i is the effect of the *i-th* treatment, β_j is the block effect and ε_{ij} is the effect of experimental error.

Experimental diets

The isoprotein base diet (35 % crude protein) in all treatments for juvenile tilapia was supplemented with *L. gibba* and *U. lactuca* in different percentages (0, 10, 15, 20 %), the treatments were identified as U0, U10, U15, U20 and L0, L10, L15, L20. They were compared with control diets supplemented with a commercial functional ingredient, mannan-oligosaccharides (MOS) (Bio-Mos® Alltech) in the same concentrations and labeled as M0, M10, M15, M20; with 0 % as the control in all treatments. The experimental diets and proximate chemical analysis are presented in Table 1. Fish were fed the diets for 5 d *ad libitum* and thereafter the feeding regime was three times a day (8:00, 14:00 and 20:00 h) at 10 % of their biomass for the first 30 days. Subsequently, from day 31 to 60, diets were provided at 8 % of fish biomass in two rations (8:00 and 20:00 h) (Zhou *et al*., 2010). The weight of the fish in each treatment was recorded every 15 days.

Evaluation of health parameters

Hematological study

Health reference values were determined using the methodology described by Horowitz *et al.* (2010) and Tukey's test, recommended by The Clinical and Laboratory

Table 1. Composition of experimental diets (g kg-1) and proximate chemical analysis.

Experimental diets												
Treatment (%)	0	10	15	20								
Fish flour (60 %)	153	153	153	153								
Soybean flour (44 %)	306	306	306	306								
Maize corn flour (8.5 %)	503	403	353	303								
Fish oil	22	22	22	22								
Vitamins and mineral premix[†]	16	16	16	16								
L. gibba or *U. Lactuca*	0	100	150	200								
MOS	0	1	1.5	2								
Proximate chemical analysis (DB)												
	L0	L10	L15	L20	U0	010	U15	U20	M0	M10	M15	M20
DM	87.4	87.6	87.4	87.5	87.5	87.4	87.4	87.6	87.4	87.5	87.5	87.4
CP	35.3	36.4	37.2	38.4	35.2	36.7	37.5	39.2	35.3	35.4	35.3	35.2
EE	2.6	2.9	3.2	3.7	3.5	3.1	3.4	3.9	2.6	2.3	2.6	2.5
CF	4.7	4.9	5.1	5.3	4.6	4.9	5.2	5.3	4.6	4.6	4.6	4.6
ASH	28.3	30.1	31.4	32	28.3	30.2	31.6	32.3	28.2	28.3	28.2	28.3
NFE	29	25.7	23.1	20.6	29.2	24.8	24.2	19.2	29.1	29.2	29.1	29.2

[†]Composition of vitamin premix (g kg^{-1} premix): vitamin A, acetate (20 000 IU g^{-1}) 5; vitamin D3 (850 000 IU g^{-1}) 0.001; DL-alpha-tocopheryl acetate (250 IU g^{-1}) 12; menadione 2. 4; thiamine-HCl 2.5; riboflavin (B2) 5; pyridoxine-HCl (B6) 4; DL Ca pantothenate 0.012; nicotinic acid 0.024; biotin 0.048; inositol 0.4; vitamin B12 4.8; folic acid 1.2; cellulose 962.62. Composition of mineral premix (g kg^{-1} premix): cobalt chloride 0.04; cupric sulfate pentahydrate 2.5; ferrous sulfate 40; magnesium sulfate heptahydrate 283.98; magnesium sulfate monohydrate 6.5; potassium iodide 0.67; sodium selenite 0.1; zinc sulfate heptahydrate 131.93; cellulose 534.28. DB: dry basis; DM: dry matter; CP: crude protein as nitrogen; EE: ether extract; CF: crude fiber; ASH: ash; NFE: nitrogen free extract.

Standards Institute (CLSI, guide C28-A3), following the technique of Fagbuaro *et al*. (2016). Fifty healthy tilapia fish of different ages (10 - 120 g) fasted for 12 h were used, anesthetized with clove essence (30 mg L^{-1}) (Sigma-Aldrich) and blood samples were taken from the caudal fin (1.5 mL) with a 21G needle in vacutainer BD EDTA K2 (United Kingdom).

Red blood cell count (E) and haematocrit (Hto) were evaluated with a Sysmex XP300 semi-automatic hematology analyzer. Hemoglobin (Hb) levels by photometry, by the cyanide-free method. Erythrocyte indices were calculated using the formulas used by Hahn-von-Hessberg (2014): mean corpuscular volume (MCV)= (haematocrit (%) × 10) /erythrocyte count (×10^6 mm^{-3}); mean corpuscular hemoglobin (MCH)= (hemoglobin (g dL^{-1}) × 10) /erythrocyte count (×10^6 mm^{-3}). Mean corpuscular hemoglobin concentration (MCHgC) = hemoglobin (g dL^{-1}) × 100/ hematocrit (%). Total protein (TP), albumin (Alb), leukocyte count (L) and glucose (G) were obtained according to Oluwalola *et al.* (2020). On days 1, 30 and 60 within the experiment, blood samples were taken from the fish for each treatment in vacutainers with anticoagulant and without anticoagulant,

as described above. Samples without anticoagulant were kept at 4 °C for later use. Samples with anticoagulant were analyzed by hematological study as described.

Lysozyme activity

The methodology applied by Soares *et al.* (2018) was used. Blood samples without anticoagulant were analyzed by commercial lysozyme test (Sigma-Aldrich, LY0100). Blood samples (90 µL) from tilapia were lysed with *Micrococcus lysodeikticus* (lyophilized cells, Sigma-Aldrich Chemical, MO, USA), at a concentration of 0.01 % w/v in reactive buffer and incubated at a temperature of 25 °C at pH 6.24. The change in absorbance at 450 nm of 0.001 per mg protein is equivalent to one unit of lysozyme, which was expressed in µg mL^{-1}.

C3 complement activity

It was determined by commercial kit (SPINREACT-1102094, C3) based on Chen *et al.* (2019). Antibodies from solution (R2) (goat serum, human anti-C3, pH 7.5, sodium azide 0.95 g L^{-1}) were combined with blood samples from tilapia that formed insoluble compounds detected through the change in absorbance at 340 nm equivalent to the concentration of C3 in the sample. A calibration curve was previously performed using the calibrator protein C3 (SPINREAC 1102004) as standard and NaCl (9 g L^{-1}) as diluent at concentrations 0.1, 0.25, 0.50, 0.75 and 1.

Productive performance

With the fortnight records of weight and feed provided to the fish, the parameters of productive performance were determined using the formulas used by Abdel-Wahab *et al.* (2016): Weight gain (WG)= final weight (g) - initial weight (g), survival (S) = 100 *(final number ÷ initial number), feed conversion factor (FCF) = feed ingested (g) ÷ weight gained (g) and protein efficiency ratio (PER) = weight gain ÷ protein consumed.

Statistical analysis

Hematologic values were analyzed with Student's *t*-test ($p \leq 0.05$) and the relationship between hematologic indices was tested by simple correlation analysis. Values obtained for lysozyme activity, C3 activity and productive performance were analyzed by one-way ANOVA ($p \leq 0.05$) and differences between treatments were analyzed post hoc with Tukey through IBM SPSS Modeler 17.0 software.

RESULTS AND DISCUSSION

Water quality in all treatments remained within desirable intervals for Nile tilapia according to El-Sayed (2020). The values of the variables monitored in the water showed no significant differences ($p \leq 0.05$) in any of the parameters temperature 27.4 ± 1.14 °C, pH 7.11 ± 0.32 nor oxygen demand 6.9 mg L^{-1}

Evaluation of health parameters

Hematological study

Although tilapia is farmed worldwide, in Latin America reports on hematological parameters for healthy tilapia are scarce (Hahn-Von-Hessberg *et al*., 2011), so they were determined in this study (Table 2). There were significant differences ($p \leq 0.05$)

Table 2. Hematological study and their reference intervals of juvenile Nile tilapia fed the different experimental diets.

	M0	M10	M15	M20	Reference interval
E (mm^3)	1.3 ± 0.03b	1.5 ± 0.06b	1.7 ± 0.05a	1.7 ± 0.03a	1.1 – 1.8
Hb (g dL^{-1})	4.6 ± 0.03d	5.7 ± 0.05c	6.8 ± 0.02b	7.9 ± 0.05a	3.3 – 7.7
Hto (%)	12.7 ± 0.03d	14.6 ± 0.03c	17.3 ± 0.03b	18.4 ± 0.04a	11.0 – 19.0
MCV (Fl)	90.0 ± 0.05d	93.0 ± 0.07c	96.0 ± 0.04b	102.0 ± 0.05a	76.5 – 104
MCH (pg)	28.5 ± 0.05c	31.2 ± 0.07b	33.3 ± 0.05b	35.1 ± 0.05a	22.5 – 37.5
MCHgC (g dL^{-1})	28.2 ± 0.08d	29.5 ± 0.05c	31.6 ± 0.08b	33.5 ± 0.07a	27.1 – 33
L (mm^3)	5150 ± 0.05d	6200 ± 0.05c	6600 ± 0.02b	6750 ± 0.04a	4000 – 6900
TP (g L^{-1})	4.5 ± 0.09d	5.9 ± 0.05c	6.3 ± 0.05b	7.4 ± 0.03a	3.4 – 7.9
ALB (g L^{-1})	2.3 ± 0.05d	2.7 ± 0.05c	3.1 ± 0.5b	4.0 ± 0.06a	2.2 – 5.0
G (mg L^{-1})	55.0 ± 0.03a	54.0 ± 0.03a	55.0 ± 0.03a	56.0 ± 0.02a	47.0 – 59.0
	U0	U10	U15	U20	
E (mm^3)	1.3 ± 0.05c	1.4 ± 0.03c	1.5 ± 0.03b	1.6 ± 0.03a	
Hb (g dL^{-1})	4.6 ± 0.03d	4.8 ± 0.05c	5.6 ± 0.05b	6.9 ± 0.02a	
Hto (%)	11.9 ± 0.03d	13.6 ± 0.02c	15.6 ± 0.04b	17.10 ± 0.03a	
MCV (Fl)	89.3 ± 0.04d	91.5 ± 0.05c	94.2 ± 0.05b	98.4 ± 0.07a	
HCM (pg)	28.4 ± 0.03d	29.6 ± 0.04c	31.3 ± 0.04b	33.8 ± 0.04a	
MCHgC (g dL^{-1})	29.0 ± 0.9c	29.3 ± 0.50c	29.8 ± 0.04b	32.7 ± 0.08a	
L (mm^3)	5200 ± 0.07d	5850 ± 0.04c	6100 ± 0.06b	6300 ± 0.05a	
TP (g L^{-1})	4.4 ± 0.03c	4.5 ± 0.03c	5.2 ± 0.05b	6.3 ± 0.05a	
ALB (g L^{-1})	2.4 ± 0.05c	3.1 ± 0.05b	3.7 ± 0.04a	4.3 ± 0.04a	
G (mg L^{-1})	55.0 ± 0.04a	55.0 ± 0.04a	56.0 ± 0.03a	55.0 ± 0.03a	
	L0	L10	L15	L20	
E (mm^3)	1.3 ± 0.03b	1.3 ± 0.05b	1.4 ± 0.03a	1.4 ± 0.02a	
Hb (g dL^{-1})	4.5 ± 0.02c	4.6 ± 0.02c	5.0 ± 0.05b	5.8 ± 0.02a	
Hto (%)	12.0 ± 0.03c	14.1 ± 0.06b	14.6 ± 0.06 b	16.5 ± 0.02a	
MCV (Fl)	90.1 ± 0.03d	91.5 ± 0.05c	93.5 ± 0.05b	96.2 ± 0.08a	
MCH (pg)	28.1 ± 0.06c	28.4 ± 0.03c	30.6 ± 0.04b	31.7 ± 0.06a	
MCHgC (g dL^{-1})	28.4 ± 0.04d	29.1 ± 0.06c	29.9 ± 0.05b	31.3 ± 0.05a	
L (mm^3)	4975 ± 0.06c	5100 ± 0.05b	5800 ± 0.05a	5900 ± 0.06a	
TP (g L^{-1})	4.6 ± 0.05c	4.8 ± 0.02c	5.3 ± 0.06b	6.0 ± 0.05a	
ALB (g L^{-1})	2.3 ± 0.04d	3.4 ± 0.05c	3.7 ± 0.05b	4.2 ± 0.05a	
G (mg L^{-1})	56.0 ± 0.03a	55.0 ± 0.03a	55.0 ± 0.03a	56.0 ± 0.03a	

± is the standard deviation (n=3). Mean values with different superscript in the same row are significant ($p \leq 0.05$). E: erythrocytes, Hb: hemoglobin, Hto: haematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoblobin, MCHgC: mean corpuscular hemoglobin concentration, L: leukocytes, TP: total proteins, ALB: albumin, G: glucose.

between treatments and different percentages of supplementation. Values increased as a function of the percentage of supplementation in all treatments. As expected, the values of the MOS controls were higher, M20 (E 1.7 mm^3, Hb 7.9 g dL^{-1}, Hto 18.4%, MCV 102 fl, MCH 35.1 pg, MCHgC 33.5 g dL^{-1}, L 6750 mm3, TP 7.4 g L^{-1}, ALB 4.0 g L^{-1}, G 56 mg L^{-1}) followed by U20 and L20 (E 1. 6 and 1.4 mm^3, Hb 6.9 and 5.8 g dL^{-1}, Hto 17.10 and 16.5%, MCV 98.4 and 96.2 Fl, MCH 33.8 and 31.7 pg, MCHgC 32.7 and 31.3 g dL^{-1}, L 6300 and 5900 mm^3, TP 6.3 and 6.0 g L^{-1}, ALB 4.3 and 4.2 g L^{-1}, G 55 and 56 mg L^{-1}, respectively) (Table 2) (Table 2). The values of the hematological parameters were within the reference interval, which meant that the treatments used did not affect the health of the fish; it stimulated the production of E and with it the production of Hb, promoting optimal oxygenation in the blood and the utilization of the nutrients in the diet. Except for the Hb value of the M20 diet, above the reference interval. Hernández *et al*. (2020) explain that because Es contain Hb (oxygen transport protein in the blood), higher levels indicate oxygen deficiency in the blood and problems in the assimilation of nutrients from food. The Hb results differ from those reported by Bittencourt *et al*. (2003) (10.5 g dL^{-1}) and Reda *et al*. (2016) (5.4 g dL^{-1}) for *O. niloticus*. El-Hawarry (2012) and Oluwalola *et al*. (2020) that blood differences occur as a function of the physiological state of the fish, their origin, water quality factors and study treatments. E, Hb and Hto had a proportional correspondence with the erythrocyte indices MCV (size), MCH (mean hemoglobin load) and MCHgC (hemoglobin color) indicating that there were no alterations and are within the health levels reported by Fagbuaro *et al*. (2016). The Hto was 3 times the Hb concentration, coinciding with that referred by El-Hawarry (2012). The production of L was promoted by both MOS and *L. gibba* and *U. Lactuca* as a function of the increase of supplementation in the experimental diets without causing affectations since all values were within the reference interval. According to Oluwalola *et al.* (2020) leukocytes participate in immune responses by fighting foreign agents. Hahn-Von-Hessberg *et al*. (2011) and Hernández *et al.* (2020) noted that leukocyte values differed in each study due to the environmental and methodological conditions of each investigation. The TP values (albumin, liver protein and globulin) were within the reference interval. They did not differ among treatments (MOS, U and L) (Table 2), however, significant differences ($p \leq 0.05$) were found in TP levels, the higher the percentage of inclusion in the experimental diets.

The above indicates that the use of these supplements in the feeding of juvenile Nile tilapia can reduce liver and nutritional problems (Fagbuaro *et al.*, 2016). Levels outside the reference interval are associated with infections, nutritional deficiencies, and stress (Reda *et al*., 2016 and Oluwalola *et al.*, 2020). It is worth mentioning that TP influence positively or negatively depending on its value on G levels, since proteins are the main source of energy and play an important role in the maintenance of blood glucose (Yildirim *et al*., 2009 and Oluwalola *et al*., 2020). The ALB and G values in the treatments of this study were different from those obtained by Fagbuaro *et al.* (2016) although they were within the reference interval, indicating that the nutrients in the experimental diets were satisfactorily absorbed and that the diets had a satisfactory

amount of protein for the fish (El-Hawarry, 2012). Hernández *et al.* (2020) and Onyia *et al.* (2015) indicated that serum biochemistry in fish can be influenced by several factors such as water temperature, feed, fish age, sex, genetics, treatments, and captivity conditions.

Lysozyme activity and complement C3 activity

Lysozyme in fish is an indicator of non-specific immune functions, functions as an opsonin and activator of the complement system and phagocytosis (Cerezuela *et al*., 2012). The complement system is a component of the immune system consisting of numerous proteins and all pathways generate factor C3, which is responsible for regulating the nonspecific and adaptive immune response (Chen *et al*., 2019). Lysozyme and C3 activities increased proportionally to tilapia growth, percentage of supplementation and elapsed days of experimentation; thus, they were more evident at day 60 in all treatments, although with significant differences ($p \leq 0.05$). Activities were higher in U20 (269 μg mL^{-1} enzyme and 122 mg dL^{-1}, respectively) than in L20 (256 μg mL^{-1} enzyme and 116 mg dL^{-1}, respectively) but not regarding the control M20 (310 μg mL^{-1} and 139 mg dL^{-1}, respectively) (Figures 1 and 2). Considering the value of M20 as 100 % maximum effect on lysozyme and C3 activity, it was observed that with the U20 diet fish reached 86 and 88% of the maximum effect, and with the L20 diet, 82 and 83 % respectively.

The behavior of the *U. lactuca* and *L. gibba* treatments was similar to the MOS control, with an increase in lysozyme and complement C3 activities that influenced hematological parameters, mainly leukocytes. Results were similar to those obtained by Soares *et al*. (2018) who with 0.2 and 0.4 % Glucan MOS in Pacu fish (*Piaractus mesopotamicus*) obtained an increase in leukocyte respiratory burst and lysozyme.

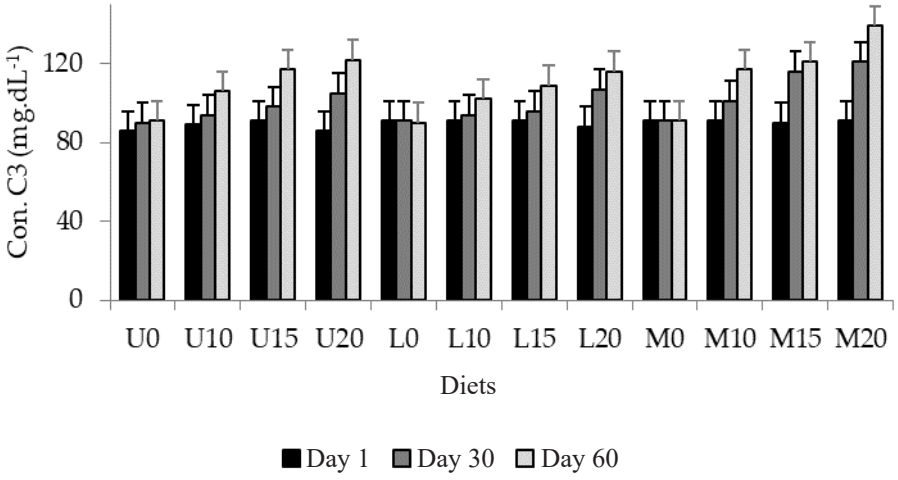

Figure 1. Comparison of complement C3 activity corresponding to experimental diets and days elapsed.

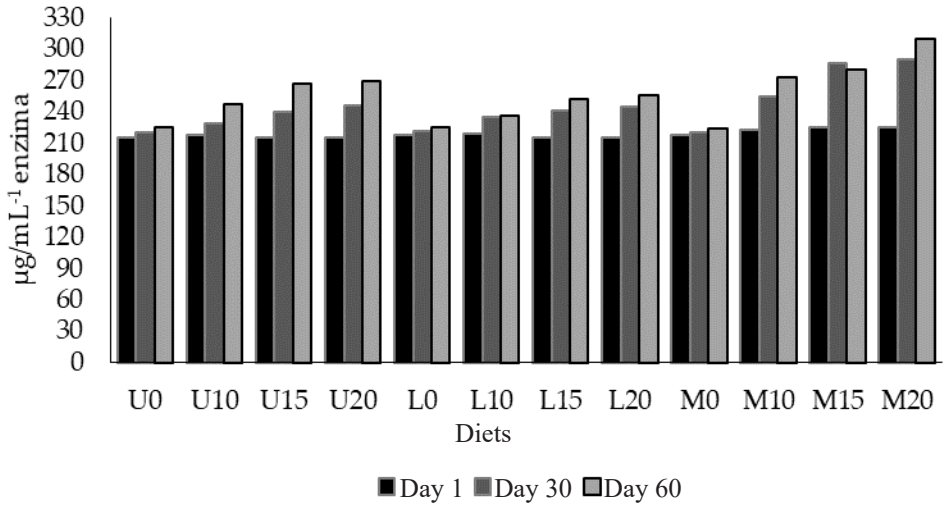

Figure 2. Comparison of lysozyme concentration corresponding to experimental diets and days elapsed.

The results obtained are in the interval obtained by Cerezuela *et al.* (2012) who used microalgae and demonstrated improvements in the immune response of fish. Those authors explained that lysozyme activity varies with age, fish size, water quality and degree of exposure to treatments.

Lin *et al.* (2011) demonstrated increased lysozyme activity and phagocytosis with MOS in *Cyprinus carpio*. The promotion of lysozyme and complement C3 activities may be related to the composition of aquatic plants rich in polysaccharides and antioxidants in their cell walls. *U. lactuca* contains ulvans, sulfated polysaccharides associated with immunomodulatory, antibacterial and antifungal functions among others (Hentati *et al*., 2020). Whereas the genus *Lemna* contains a wide variety of galacturonan polysaccharides, proteins and phenolic compounds (Zhao *et al*., 2014). There was a directly proportional relationship of complement C3 activity with leukocytes, it may be that these are stimulated by polysaccharides in both aquatic plants. Chen *et al.* (2019) referred that leukocytes form the first line of defense, an anti-attack complex against foreign bodies by activating complement C3.

Productive performance

Diets supplemented with *L. gibba* and *U. lactuca* were highly accepted by the Nile tilapia fish, Yildirim *et al*. (2009) reported that it is probably because of their odor and succulent taste. The mean final weight of fish fed the diets supplemented with *L. gibba* and *U. lactuca* was significantly higher ($p \leq 0.05$) than their controls (L0 and U10, respectively) and the MOS control diets. The L15 diet presented the best productive performance values (FP 131 g, PER 3.08 and FCF 1.5) followed by the U15 diet (127 g, 3.11 and 1.78, respectively) and the M15 control (112 g, 270 and 1.6, respectively). In

all treatments, survival was 100 % (Table 3) Although the U20 diet gave the best health results at the cellular and humoral levels, growth was negatively affected. Abdel-Wahab *et al.* (2016) explained that the decrease in growth at high levels of Ulva meal (30 %) could be due to the high fiber content and its possible effects on protein and dry matter digestibility. The variation in chemical constituents of aquatic plants such as *U. lactuca* is influenced by the environment in which they are found (Suryaningrum and Samsudin, 2020).

The diets with MOS had no effect on fish growth, which is in agreement with that reported by Soares *et al.* (2018), who explained that the effect of MOS on growth in fish depends on the species to which it is supplied, the time of application and the temperature of the environment. The better productive performance of L15 was partly due to the contribution of protein and amino acids from aquatic plants, mainly growth-promoting lysine, and methionine (Yildirim *et al*., 2009; Aguilera *et al*., 2018) (Table 4). Protein from both *L. gibba* and *U. lactuca* was used as an energy source for growth. *L. gibba* has shown to contribute to the growth of several fish species when part of the fish meal is substituted (El-Sayed, 2019). González *et al.* (2013) reported increased growth of tilapia fry when 12 % *Lemna trinervis* was included in the diet. El-Shafai *et al.* (2004) replaced 20 to 40 % of fish meal with *Lemna minnor* meal in fresh and dehydrated form, both of which favored growth, PER and the best FCF (1.4) in tilapia. *U. lactuca* has also shown to promote growth when included at 20 to 30 % in the diet of African catfish *Clarias gariepinus* (Abdel-Wahab *et al*., 2016) and at 5 and 10 % in juvenile fish *Scatophagus argus* (Yangthong and Ruensirikul, 2020). In tilapia diet it has been included at 10 %, although inclusion up to 20 % is suggested, with satisfactory

Table 3. Comparison of zootechnical parameters of juvenile Nile tilapia fed with the experimental diets (mannan-oligosaccharides, *Ulva lactuca* and *Lemna gibba*) at different percentages of supplementation (0, 10,15 and 20).

	IP(g)	FW (g)	S (%)	PER	FCF
M0	9.37 ± 0.25	104.03 ± 1.50c	100	2.71 ± 0.04c	1.42 ± 0.03c
M10	9.43 ± 0.31	110.17 ± 1.02b	100	2.88 ± 0.03b	1.45 ± 0.05c
M15	9.43 ± 0.32	113.77 ± 1.56b	100	2.98 ± 0.04b	1.55 ± 0.05b
M20	9.40 ± 0.20	119.37 ± 2.15a	100	3.14 ± 0.06a	1.60 ± 0.06a
U0	9.37 ± 0.25	103.03 ± 1.56c	100	2.68 ± 0.04c	1.42 ± 0.03a
U10	9.37 ± 0.23	111.97 ± 2.63b	100	2.93 ± 0.08b	1.52 ± 0.05b
U15	9.30 ± 0.20	127.03 ± 1.72a	100	3.36 ± 0.05a	1.38 ± 0.03b
U20	9.40 ± 0.10	123.93 ± 2.70a	100	3.27 ± 0.08a	1.64 ± 0.02b
L0	9.33 ± 0.12	104.37 ± 1.98c	100	2.71 ± 0.05c	1.42 ± 0.03a
L10	9.23 ± 0.15	110.33 ± 1.66b	100	2.89 ± 0.05b	1.52 ± 0.02b
L15	9.33 ± 0.25	104.37 ± 1.98c	100	2.72 ± 0.05c	1.40 ± 0.02c
L20	9.40 ± 0.17	117.83 ± 1.31a	100	2.95 ± 0.05a	1.59 ± 0.05c

± is the standard deviation (n=3). Mean values with the same level of supplementation and different superscript in the same column are significant ($p \leq 0.05$). IW: initial weight; FW: final average weight; S: survival; PER: protein efficiency ratio; FCF: feed conversion factor.

Table 4. Chemical composition (dry basis) and amino acids present in *Ulva lactuca* and *Lemna gibba.*

Component	*Lemna gibba*	*Ulva lactuca*
Ash (%)	20.10	33.07
Crude protein %	21.5	17.2
Lipids %	4.45	1.7
Nitrogen free extract %	32.4	38.34
Amino acidS composition		
Arginine	0.003	0.004
Histidine	0.001	0.001
Isoleucine	1.569	4.57
Leucine	0.003	9.33
Lysine	22.33	9.24
Methionine	8.57	5.92
Threonine	0.21	4.76
Valine	2.23	4.01
Alanine	27.80	9.77
Glycine	1.76	6.11

(Aguilera *et al*., 2018).

results in growth, feed utilization and survival rate (Suryaningrum and Samsudin, 2020).

CONCLUSIONS

All diets supplemented with *Lemna gibba* and *Ulva lactuca* showed an effect on the cellular (hematological study) and humoral parameters evaluated (lysozyme and complement C3 activity), in regard to the control diets (L0 and U0). However, statistically the effect was greater in L20 and U20. According to the hematological study, *U. lactuca* and *L. gibba* contributed to improved oxygen transport in the blood, increased leukocyte, increased lysozyme and C3 activity, without affecting the nutritional quality of the diet or affecting the health of the fish. The U20 diet showed the greatest significant effect on health parameters and L15 showed the best results on production.

U. lactuca and *L. gibba* can be used as a functional ingredient in a proportion of 15 % for the production of Nile tilapia juvenile feed, with favorable effects on health and growth. *U. lactuca* can be included in the diet of juvenile tilapia up to 20 %, because it favors a higher activity of lysozyme and C3 complement, after verifying the content of *Ulva* fiber since it varies according to its habitat and can promote negative growth.

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REFERENCES

Abdel-Wahab AAW, El-Sayed MIY, Nasser AA. 2016. Potential use of green macroalgae *Ulva lactuca* as a feed supplement in diets on growth performance, feed utilization and body composition of the African catfish, *Clarias gariepinus*. Saudi Journal of Biological Sciences 23 (3): 404–409. https://doi.org/10.1016/j.sjbs.2015.11.010

Aguilera Morales ME, Canales Martínez M, Ávila González E, Flores Ortíz CM. 2018. Nutrients and bioactive compounds of the *Lemna gibba* and *Ulva lactuca* as possible ingredients to functional foods. Latin American Journal of Aquatic Research 46 (4): 709–716. http://dx.doi.org/10.3856/vol46-issue4-fulltext-8

Beltrán de Heredia, MR. 2016. Alimentos funcionales. Farmacia profesional 30 (2): 12–14.

Bittencourt NL, Molinari LM, Scoaris DO, Pedroso RB, Nakamura CV, Nakamura TU, Filho BA, Filho BP. 2003. Haematological and biochemical values for Nile tilapia *Oreochromis niloticus* cultured in semi-intensive system. Acta Scientiarum: Biological Sciences 25 (2): 385–38.

Cerezuela R, Guardiola FA, Meseguer J, Esteban MA. 2012. Enrichment of gilthead seabream (*Sparus aurata* L.) diet with microalgae: effects on the immune system. Fish Physiology Biochemical 38 (6): 1729–1739. https://dx.doi.org/10.1007/s10695-012-9670-9

Chen DD, Li JH, Yao YY, Zhang YA. 2019. *Aeromonas hydrophila* suppresses complement pathways via degradation of complement C3 in bony fish by metalloprotease. Fish and Shellfish Immunology 94: 739–745. https://dx.doi.org/10.1016/j.fsi.2019.09.057

Código Sanitario para Animales Acuáticos. 2019. Organización mundial de sanidad animal (OIE). https://www.oie.int/es/normas/codigo-acuatico/acceso-en-linea/ (Recuperado: diciembre 2021).

El-Hawarry WN. 2012. Biochemical and non-specific immune parameters of healthy Nile tilapia *Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*) and their interspecific hybrid (male *O. aureus* × female *O. niloticus*) maintained in semi-intensive culture system. Online Journal of Animal and Feed Research 2 (1): 84–88.

El-Sayed A-F M. 2020. Tilapia Culture. 2nd Edition. Academic Press: Oxfordshire, U.K. 348 p. https://doi.org/10.1016/C2017-0-04085-5

El-Shafai SA, El-Gohary FA, Verreth JAJ, Schrama JW, Gijzen HJ. 2004. Apparent digestibility coefficient of duckweed (*Lemna minor*), fresh and dry for Nile tilapia (*Oreochromis niloticus* L.). Aquaculture Research 35 (6): 574–586. https://doi.org/10.1111/j.1365-2109.2004.01055.x

FAO (Organización de las Naciones Unidas para la Alimentación y la Agricultura). 2019. Contribución de la acuicultura a la erradicación del hambre, la obtención de suministros alimentarios y la promoción de buenas prácticas higiénicas y dietéticas. Organización de las Naciones Unidas para la Alimentación y la Agricultura (FAO). Comité de pesca, subcomité de acuicultura. Trondheim (Noruega). COFI:AQ/X/2019/6/Rev.1. 18 p. http://www.fao.org/3/na220es/na220es.pdf (Recuperado: septiembre 2021).

Fagbuaro O, Oladimeji IA, Adeyinka FA. 2016. Heamatological and serum biochemical profile of Nile tilapia, *Oreochromis niloticus* from Ero dam in Ikun Ekiti, Ekiti state Nigeria. American Journal of Research Communication 4 (4): 192–205. http://www.usa-journals.com/wp-content/uploads/2016/04/Fagbuaro_Vol44.pdf [ISSN: 2325-4076] (Recuperado: noviembre 2021).

González S R, Romero C O, Valdivié N M, Ponce P J. 2013. Lenteja de agua una opción en dietas para tilapia roja. Revista AquaTIC 38: 85–93.

Hahn-von-Hessberg C, Grajales-Quintero A, Gutiérrez-Jaramillo AV. 2011. Parámetros hematológicos de Tilapia nilótica (*Oreochromis niloticus*, Linneaus 1757) con peso entre 250 g y 350 g, en el Centro Experimental Piscícola de la Universidad de Caldas. Veterinaria y Zootecnia 5 (1): 47–61.

Hentati F, Tounsi L, Djomdi D, Pierre G, Delattre C, Ursu AV, Fendri I, Abdelkafi S, Michaud P. 2020. Bioactive polysaccharides from seaweeds. Molecules 25 (14): 3152. https://doi.org/10.3390/molecules25143152

Hernández C, Olmeda-Guerrero L, Chávez-Sánchez MC, Ibarra-Castro L, Gaxiola-Cortés G, Martínez-Cárdenas L. 2020. Nutritional evaluation of canola meal as fish meal replacement for juvenile spotted rose snapper (*Lutjanus guttatus*): Effects on growth performance, hematological parameters, body composition, and nutrient digestibility. Animal Feed Science and Technology 269: 114683. https://doi.org/10.1016/j.anifeedsci.2020.114683

Horowitz GL, Altaie S, Boyd JC, Ceriotti F, Garg U, Horn P, Pesce A, Sine HE, Zakowski J. 2010. EP28-A3c: Defining, Establishing and Verifying Reference Intervals in the Clinical

Laboratory; Approved Guideline. Clinical and Laboratory Standards Institute. 3rd Edition. Wayne, PA, USA. 59 p.

Lin S, Pan Y, Luo L, Luo L. 2011. Effects of dietary β-1,3-glucan, chitosan or raffinose on the growth, innate immunity and resistance of koi (*Cyprinus carpio* Koi). Fish and Shellfish Immunology 31 (6): 788–794. https://doi.org/10.1016/j.fsi.2011.07.013

NRC (National Research Council). 2011. Nutrient Requirements of Fish and Shrimp. The National Academy Press: Washington, DC, USA. 392 p. https://doi.org/10.17226/13039

Oluwalola OI, Fagbenro OA, Adebayo OT. 2020. Haematological and serum biochemical profiles of Nile tilapia, *Oreochromis niloticus* from different culture enclosures. International Journal of Fisheries and Aquatic Studies 8 (3): 489–493.

Onyia LU, Diyaware MY, Michael KG, Musa M, Ochokwu IJ. 2015. Comparison of haematological indices, blood group and genotype of *Clarias gariepinus* (Burchell, 1822) and *Clarias anguillaris* (Linneaus, 1758). Journal of Fisheries and Aquatic Science 10 (5): 392–399. https://doi.org/10.3923/jfas.2015.392.399

Reda RM, Mahmoud R, Selim KM, El-Araby IE. 2016. Effects of dietary acidifiers on growth, hematology, immune response and disease resistance of Nile tilapia, *Oreochromis niloticus*. Fish and Shellfish Immunology 50: 255–262. https://dx.doi.org/10.1016/j.fsi.2016.01.040

Soares MP, Oliveira FC, Cardoso IL, Urbinati EC, Meldau de Campos C, Hisano H. 2018. Glucan-MOS® improved growth and innate immunity in pacu stressed and experimentally infected with *Aeromonas hydrophila*. Fish and Shellfish Immunology 73: 133-140. https://dx.doi.org/10.1016/j.fsi.2017.11.046

Suryaningrum LH, Samsudin R. 2020. Nutrient digestibility of green seaweed *Ulva* meal and the influence on growth performance of Nile tilapia (*Oreochromis niloticus*). Emirates Journal of Food and Agriculture 32 (7): 488–494. https://doi.org/10.9755/ejfa.2020.v32.i7.2131

Tirado-Estrada G, Ramos-Mijangos LM, Miranda-Romero LA, Tirado-González DN, M. Salem AZM, Mlambo V, Medina-Cuéllar SE, González-Reyes M, Barababosa P A. 2018. Potential impacts of dietary *Lemna gibba* supplements in a simulated ruminal fermentation system and environmental biogas production. Journal of Cleaner Production 181: 555–561. https://doi.org/10.1016/j.jclepro.2018.01.120

Yangthon M, Ruensirikul J. 2020. Feed intake stimulation of juvenile spotted scat (*Scatophagus argus* Linnaeus, 1766) using dietary seaweed supplementation (*Ulva* sp.). Aquaculture 529: 735626. https://doi.org/10.1016/j.aquaculture.2020.735626

Yildirim Ö, Ergün S, Yaman S, Türker A. 2009. Effects of two seaweeds *(Ulva lactuca* and *Enteromorpha linza*) as a feed additive in diets on growth performance, feed utilization, and body composition of Rainbow trout (*Oncorhynchus mykiss*). Kafkas Universitesi Veteriner Fakultesi Dergisi 15 (3): 455–460.

Yuji-Sado R, Raulino-Domanski F, Freitas PF, Baioco-Sales F. 2015. Growth, immune status and intestinal morphology of Nile tilapia fed dietary prebiotics (mannan oligosaccharides-MOS). Latin American Journal of Aquatic Research 43 (5): 944–952. https://dx.doi.org/10.3856/vol43-issue5-fulltext-14

Zhao X, Moates GK, Wellner N, Collins SRA, Coleman MJ, Waldron KW. 2014. Chemical characterisation and analysis of the cell wall polysaccharides of duckweed (*Lemna minor*). Carbohydrate Polymers 111: 410–418. https://doi.org/10.1016/j.carbpol.2014.04.079

Zhou QC, Buentello JA, Gatlin III DM. 2010. Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture 309 (1-4): 253–257. https://dx.doi.org/10.1016/j.aquaculture.2010.09.003