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Effect of prebiotics on the growth performance, haematological, biochemical, and histological parameters of African catfish (*Clarias gariepinus*) in recirculating aquaculture system

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Abstract: In this study, African catfish (*Clarias gariepinus*) was investigated under controlled conditions in recirculating aquaculture system (RAS). The experiment tested the effects of three different dietary prebiotics (FOS: fructooligosaccharide, GOS: galactooligosaccharide, and MOS: mannanoligosaccharide) in two levels (1 g/kg and 2 g/kg) and a control diet on the growth performance, survival rate, haematological and biochemical parameters, and small intestinal and liver histology of the African catfish. Better growth results were obtained in MOS (2 g/kg) supplementation, and there was a statistical difference ($P < 0.05$) between the MOS (2 g/kg) and the control group. Haematological parameters (erythrocyte sedimentation rate, red blood cell, white blood cell, lymphocyte, neutrophil, and glucose) were similar among all groups ($P > 0.05$) while some (alanine aminotransferase, aspartate aminotransferase enzymes, and monocyte) were significantly different ($P < 0.05$). The MOS (2 g/kg) group had longer villi length values than the control group. There was no difference among the groups in terms of small intestine histomorphology and liver tissues in fish. According to the results of this experiment, it can be said that MOS addition has a positive effect on growth parameters and could improve health conditions in *Clarias gariepinus* culture.

Key words: Clarias, prebiotic, growth, haematology, histology

1. Introduction

The world population is expected to reach 9 billion by 2050 [1]. Safe and nutritious production techniques are among the priorities to meet the increasing diets needs in the world [2]. Aquaculture is one of the fastest growing food production sectors and accounts for 16.6% of the animal protein consumed worldwide [3]. It is the strongest candidate to meet the growing food demand in the future [3,4].

Increased stress in intensive aquaculture conditions may cause host resistance to bacterial, viral, and parasitic diseases in the environment, resulting in the formation of diseases in a short time [5]. The use of subtherapeutic doses of antibiotics, disinfectants, and other chemicals to improve growth and disease protection rates in aquaculture has increased with the expansion of aquaculture activities [6]. However, antibiotic use has raised controversies in terms of risks and environmental impacts [7]. The use of antibiotics for protection may lead to the development of resistance to antibiotics in microorganisms and may also have a harmful effect on the aquatic environment and human health. In this framework, the use of functional feed additives has improved to increase the resistance of species cultured against diseases and stress resistance. Some of the functional feed additives are probiotics, prebiotics, enzymes, and immunostimulants [8]. Studies have demonstrated that the use of alternative functional ingredients to promote healthy growth in aquaculture and to increase the quantity and quality of products has gained importance [9–13]. Prebiotics are one of the major feed additives and may have positive effects on growth performance, survival rate, feed conversion ratio, digestion, gastrointestinal (GI) enzyme activity, and the immune system [14]. Fructooligosaccharides (FOS), mannanoligosaccharides (MOS), and galactooligosaccharides (GOS) are common prebiotics used in diets for fish. Mannanoligosaccharide (MOS), a prebiotic derived from baker's yeast (*Saccharomyces*

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cerevisiae), has been reported to be effective in aquaculture [15–18]. Fructooligosaccharide (FOS or oligofructose), which is obtained by the enzymatic hydrolysis of inulin, is a fructan and is found in many common foods such as garlic, onions, artichokes, and asparagus. FOS has been the subject of studies as a prebiotic in aquaculture [19–21]. Another carbohydrate-based food component is galactooligosaccharide (GOS), and it can improve healthy physiological activities. GOS can be used to reduce the number of potentially pathogenic bacteria, to facilitate the normal functioning of the gut, and to prevent infections [22]. It can also provide benefits in stimulating the absorption of certain minerals and reducing lipid content in the blood [23].

The use of natural substances that have growth-enhancing effects on feed has been gaining scientific value in recent years [24,25]. Although there are many studies on the effects of MOS, FOS, and GOS in different fish species, there is still a lack of knowledge about *Clarias gariepinus*. Investigating the effects of these prebiotics on *C. gariepinus* culture conditions will contribute to aquaculture. Therefore, this study aimed to investigate the effects of MOS, FOS, and GOS on the growth performance and the haematological, biochemical, and histological parameters of the African catfish (*Clarias gariepinus*) in recirculating aquaculture systems.

2. Materials and methods

2.1. Broodstock transport and experimental design

The study was carried out in the recirculating aquaculture system (RAS) at the Fisheries Research and Application Unit of the Agricultural Faculty of Ankara University, Turkey. The African catfish, *Clarias gariepinus* broodstocks were obtained from Antakya (in Hatay, Turkey) and were transported from Hatay to Ankara in 2 80-L plastic transport tanks under continuous aerated conditions. Eight broodstock fish (4♀: 1194.50 ± 45.97 g and 4♂: 996.25 ± 16.17 g) were stocked in tanks with a density of 1 fish/50 L for the acclimation to laboratory conditions of RAS (after the 0.8–1% saltwater disinfection bath). The pituitary hormone injection (6 mg/kg) was applied 8–9 h before the stripping and fertilisation of mature eggs. Eggs from the broodstocks (under light anaesthesia with Eugenol 22–25 mg/L) were collected by dry strip method. Since spermatozoa could not be obtained from mature male fish, the testes were removed after testicular sperm was collected according to the insemination protocol (with euthanasia by deep anaesthesia using Eugenol 100 mg/L) [26,27]. Incubation (the eggs adhering to the raffia) was continued for up to 21 ± 1 h at a water flow of 2 L/min (constant water flow pump, Sparus Pentair Aquatics®, California, USA) with a pH of 7.6 ± 0.2 and a water temperature of 27.8 ± 1 °C in 40-L tanks.

For the experiment, the larvae (0.20–0.23 g) were stocked in the tanks (40 larvae/40 L). The effects of two different doses (1 and 2 g/kg) of 3 different feed additives (fructooligosaccharide, galactooligosaccharide, and mannanoligosaccharide) were tested by forming 7 groups with triplicates each. These groups were designed as control, 1 g/kg and 2 g/kg fructooligosaccharide (FOS1 and FOS2), 1 g/kg and 2 g/kg galactooligosaccharide (GOS1 and GOS2), and 1 g/kg and 2 g/kg mannanoligosaccharide (MOS1 and MOS2). Feeding was performed three times daily (08.00, 13.00, and 18.00) ad libitum in 12 h light and 12 h dark photoperiod conditions.

During the study, water exchange was applied as 7% weekly and water quality parameters were maintained in optimal conditions for *Clarias gariepinus* (pH: 7.5 ± 0.4, dissolved oxygen: 6.9 ± 0.5, water temperature: 26.5 ± 1.0) [28].

2.2. Feed and feed additives

In the experiment, the commercial diet in appropriate sizes (Abalıoğlu Feed Ltd., Turkey) was used as the basal feed. Table 1 presents the nutrient component contents of the food. As the prebiotic feed additives, fructooligosaccharide (FOS, Inulin, Encore Technologies, Plymouth, MN, USA), galactooligosaccharide (GOS, Vivinal GOS, Friesland Foods Domo, Zwolle, The Netherlands), and mannanoligosaccharide (MOS, Bio-Mos, Alltech Inc., Nicholasville, KY, USA) were used. Different prebiotic doses were added to the ground commercial feed. A suitable amount of distilled water was added to obtain dough consistency, and it was passed through a pressure pellet machine of different sizes and then dried at 35 °C. The dried repelleted feed was stored in the refrigerator (+4 °C).

2.3. Growth parameters

The final live weight (FLW) and the final total length (FTL) measurements of the fish were performed under light anaesthesia conditions. The growth parameters were calculated using the following formulas (FW: final weight, IW: initial weight, t: 92 days).

Live weight gains (LWG) = FW (g) – IW (g)

Specific growth rate (SGR) = (Ln FW - Ln IW) / t ×100

Feed conversion ratio (FCR) = total given feed (g) / weight gain (g)

Survival rate (SR%) = the number of live fish at the end of the trial / the number of live fish at the beginning of the experiment × 100

2.4. Haematological parameters

The fish (two fish were sampled for each replicates, N = 6) were deeply anaesthetized with 100 mg Eugenol L^{-1}. Blood samples were taken from the caudal region of the fish (dorsal aorta) with an anticoagulant syringe during anaesthesia. The researchers used a 5-mL plastic injector

Table 1. The proximate composition of the diet.

Content	%
Crude protein	48
Crude lipid	18
Crude ash	12
Crude cellulose	2
Humidity	15

with a 21-G needle treated with commercial heparin (Nevparin) for the blood collection. The erythrocyte sedimentation rate, erythrocyte cell count, leukocyte cell count, and differential leukocyte cell numbers of the blood samples were determined. The micro-Wintrobe method was used to determine the erythrocyte sedimentation rate (ESR) [29,30]. For the determination of erythrocyte (RBC) and leukocyte (WBC) cell count, the blood was drawn up to the line 1 of the erythrocyte/leukocyte pipette with the Natt-Herrick solution and the cells stained under the light microscope were counted using the Nauber slide. The numbers of cells were calculated using the conventional formula (cell number determined × dilution ratio × 4000 = cell number mm^{-3}). May-Grünwald and Giemsa staining were performed to determine the leukocyte cell types (monocytes, lymphocytes, and neutrophils). Cell types identified from the stained blood smears were counted (at 1000× magnification under light microscope) and their ratio to the total leukocyte count was calculated from 200 cells per blood smear slide.

2.5. Glucose, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) enzyme analysis

Blood glucose meter was used to determine the blood glucose level (Strip 06454011, Roche Accu-Check Performa Nano). The alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes were measured calorimetrically from plasma with a kit (Abcam Cambridge, UK) in 96-well microplates [31].

2.6. Hepatosomatic and viscerosomatic index and histological analysis

At the end of the experiment (according to the euthanasia protocol in deep anaesthesia condition), the internal organs and the liver were removed and measured. The data were used to calculate the hepatosomatic index (HSI) and the viscerosomatic index (VSI) (HSI% = liver weight (g) / FW (g) × 100 and VSI% = internal organ weight (g) / FW (g) × 100).

For the histological examination, small intestine tissue samples (distal, after the pyloric region of the intestinal channel) were fixed in buffered formaldehyde solution. After 24 h of fixation, paraffin blocks were prepared following dehydration and transparency. Tissues (4–6 μm) were removed using a rotary-type microtome (Thermo Shandon, Germany). At the end of the deparaffinisation, the sections were stained with haematoxylin and eosin. The villus lengths were measured under the light microscope (Leica CM40) using the images obtained by the microphotograph and measurement technique [32].

2.7. Proximate analysis

Proximate analysis including the dry matter, crude ash, and protein was performed for the African catfish (three fish each triplicate) [33]. Lipid values were analysed freshly according to the conventional technique [34].

2.8. Statistical analysis

One-way ANOVA was used to determine the effects of different oligosaccharides on growth performance, haematological parameters, glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST) enzyme analysis, villi length, and proximate analysis data evaluations. The data for the groups were given as mean ± standard deviation. The comparisons were made by using DUNCAN analysis results at the 0.05 significance level. IBM SPSS Software 23 software was used for statistical analysis.

3. Results

3.1. Growth

In the experiment, the fish with the initial live weights of 0.20–0.23 g and a total length of 2.96–3.00 cm were fed with different oligosaccharide-added feeds for 92 days. The fish fed with prebiotic-supplemented food clearly showed a better growth performance. The highest final weight was obtained in the MOS2 group and the lowest final weight was acquired in the control group ($P < 0.05$). FOS1, FOS2, GOS1, GOS2, and MOS2 groups reached higher values than that of the control group in terms of the final total length ($P < 0.05$). The best survival rates were recorded in the GOS1, GOS2, MOS1, and MOS2 groups. There was no difference among the groups in terms of the specific growth rate. The best feed conversion ratio was obtained in the MOS2 group ($P < 0.05$). There was no significant difference in the groups regarding the hepatosomatic (HSI) and viscerosomatic index (VSI) data ($P > 0.05$) (Table 2).

3.2. Haematological and biochemical parameters

There was a difference in the number of monocytes among the groups. The monocyte counts of the FOS2, GOS2, MOS1, and MOS2 groups were higher than those of the control group, FOS1, and GOS1 groups ($P < 0.05$).

ESR, WBC, RBC, lymphocyte, and neutrophil values were not significantly different between the experimental groups and the control group. Among the biochemical parameters, glucose levels were higher in the control

group than all the experimental groups (P < 0.05). Alanine aminotransferase was different among the groups, and the highest value was obtained in the MOS1 group. The levels of the aspartate aminotransferase enzyme were lower in the experimental groups compared to the control group (P < 0.05). The AST/ALT ratio was lower than 1 in all the groups and except for the GOS2 group, all the experimental groups were different from the control group data (P < 0.05) (Table 3).

3.3. Histological findings

3.3.1. Villi length

The MOS2 group had the highest villi length (P < 0.05) among the control groups and the other groups (Table 4).

3.3.2. Liver and intestine histology

The tested small intestinal tissues showed normal characteristics as basic, mucoidal, columnar epithelia, submucosal eosinophilic granulated cells, few goblet cells,

Table 2. Effect of different oligosaccharides on growth parameters.

	Control	FOS1	FOS2	GOS1	GOS2	MOS1	MOS2
IW	0.22±0.06^{a}	0.21±0.06^{a}	0.22±0.08^{a}	0.23±0.08^{a}	0.21±0.06^{a}	0.21±0.03^{a}	0.20±0.05^{a}
FW	19.54±2.28^{a}	21.35±1.68^{b}	21.66±2.41^{bc}	21.73±2.15^{bc}	21.85±2.85^{bc}	21.89±2.48^{bc}	23.38±1.77^{c}
ITL	2.96±0.24^{a}	2.96±10.24^{a}	2.99±0.30^{a}	3.00±0.30^{a}	2.97±0.25^{a}	2.99±0.33^{a}	2.99±0.29^{a}
FTL	17.55±0.71^{a}	18.27±1.52^{ab}	18.93±1.16^{bc}	19.07±1.50^{bc}	18.63±1.35^{bc}	18.29±1.07^{ab}	19.58±1.35^{c}
WG	19.32±1.13^{a}	21.14±0.67^{ab}	21.43±0.64^{ab}	21.50±1.20^{ab}	21.63±1.80^{b}	21.68±1.67^{b}	23.18±0.72^{b}
DWG	0.21±0.01^{a}	0.23±0.02^{ab}	0.23±0.02^{abc}	0.24±0.02^{bc}	0.23±0.02^{abc}	0.24±0.02^{bc}	0.25±0.01^{c}
SGR	4.88±0.17^{a}	5.02±0.08^{a}	4.98±0.12^{a}	4.96±0.12^{a}	5.04±0.23^{a}	5.04±0.17^{a}	5.17±0.19^{a}
FCR	1.84±0.12^{d}	1.77±0.08^{cd}	1.62±0.08^{abc}	1.72±0.07^{bcd}	1.55±0.15^{ab}	1.63±0.15^{abc}	1.49±0.04^{a}
SR	85.00±4.33^{ab}	80.00±4.33^{a}	85.00±2.50^{ab}	95.00±2.50^{c}	90.00±2.50^{bc}	90.00±4.33^{bc}	95.00±2.50^{c}
HSI	1.870±0.134^{a}	1.843±0.074^{a}	1.830±0.164^{a}	1.875±0.178^{a}	1.782±0.135^{a}	1.8443±0.148^{a}	1.808±0.081^{a}
VSI	9.943±0.671^{a}	9.607±0.594^{a}	9.582±0.756^{a}	9.362±0.687^{a}	9.501±1.223^{a}	9.240±0.369^{a}	9.421±1.312^{a}

*The lower-case superscripts indicate differences between the groups (P < 0.05). The trial period was 92 days.
IW: Initial weight (g), FW: Final weight (g), ITL: Initial total length (cm), FTL: Final total length (cm), WG: Weight gain (g), DWG: Daily weight gain (g), SGR: Specific growth rate (%), FCR: Feed conversion ratio, SR: Survival rate (%). HSI: Hepatosomatic index, VSI: viscerosomatic index. HSI % = Liver weight (g) / live weight (g) × 100, VSI % = Internal organ weight (g) / live weight (g) × 100.

Table 3. Effect of different oligosaccharides on haematological and biochemical parameters.

	Control	**FOS1**	**FOS2**	**GOS1**	**GOS2**	**MOS1**	**MOS2**
ESR (mm/hr)	1.78±0.34^{a*}	1.64±0.25^{a}	1.58±0.39^{a}	1.61±0.33^{a}	1.52±0.24^{a}	1.53±0.23^{a}	1.51±0.31^{a}
RBC (×10^{6} mm^{-3})	2.66±0.46^{a}	2.93±0.41^{a}	2.99±0.45^{a}	2.97±0.39^{a}	3.01±0.55^{a}	2.99±0.35^{a}	3.08±0.32^{a}
WBC (×10^{3} mm^{-3})	23.33±2.50^{a}	23.89±2.57^{a}	24.67±2.24^{a}	24.67±1.41^{a}	23.56±2.30^{a}	24.11±2.03^{a}	24.89±2.37^{a}
Monocyte (%)	7.22±0.83^{a}	8.11±1.36^{ab}	8.67±0.71^{b}	8.33±1.00^{ab}	8.56±1.42^{b}	8.67±1.00^{b}	8.89±1.45^{b}
Lymphocyte (%)	64.00±3.20^{a}	64.78±3.31^{a}	65.33±3.32^{a}	65.22±2.39^{a}	65.78±1.56^{a}	65.56±2.07^{a}	65.89±0.93^{a}
Neutrophil (%)	23.11±1.96^{a}	23.56±2.40^{a}	23.89±1.96^{a}	23.89±3.59^{a}	24.22±2.11^{a}	24.67±1.66^{a}	24.11±1.54^{a}
Glucose (mg/mL)	125.22±7.82^{b}	120.78±4.09^{a}	119.00±4.15^{a}	118.89±4.31^{a}	120.11±2.42^{a}	118.89±2.09^{a}	117.89±3.48^{a}
ALT (mU/mL)	20.80±1.11^{bc}	20.59±0.94^{a}	20.24±0.55^{ab}	20.74±1.00^{abc}	19.69±1.34^{a}	21.73±1.04^{c}	20.26±0.95^{ab}
AST (mU/mL)	20.22±1.11^{c}	18.28±1.44^{ab}	18.26±1.08^{ab}	17.93±1.07^{ab}	18.30±1.16^{ab}	18.58±0.76^{b}	17.26±0.65^{a}
AST/ALT	0.97±0.05^{c}	0.89±0.08^{ab}	0.90±0.06^{ab}	0.87±0.07^{a}	0.93±0.04^{bc}	0.86±0.07^{a}	0.85±0.05^{a}

*The lower-case superscripts indicate differences between the groups (P < 0.05).
ESR: Erythrocyte sedimentation rate, RBC: Erythrocyte, WBC: Leucocytes, Leucocytes cell types: monocytes, lymphocytes, neutrophil, ALT: alanine aminotransferase, and AST: aspartate aminotransferase enzyme (mU/mL).

Table 4. Effect of different oligosaccharides on small intestine villi length.

	Control	FOS1	FOS2	GOS1	GOS2	MOS1	MOS2
VL	1360.88±293.267[a*]	1409.59±354.41[a]	1479.38±288.42[ab]	1451.99±185.90[ab]	1500.00±227.58[ab]	1512.22±202.48[ab]	1605.19±196.20[b]

*The lower-case superscripts indicate differences between the groups ($P < 0.05$).
*VL: villus length (*μm*).*

muscular mucosa, areolar connective tissue, elastic fibres layers, and outer parts as a circular layer and a longitudinal layer of muscular externa (Figure 1).

The common sinusoidal architecture was seen in the examined *Clarias gariepinus* hepatic parenchyma. Liver endothelial cells, cell membrane, and very prominent nuclei were distributed irregularly between the hepatocytes as polygonal cells. Functional phagocytic cells were observed in the sinusoids. The sinusoids are covered by endothelial cells which consist of elongated dark nuclei and Kupffer cells. The Kupffer cells were showed as stellate-shaped and broad ovoidal dark blue nucleus was in the lumen of liver sinusoids. As seen in cultured fish fed with formulated diet, catfish liver tissues contained natural lipid accumulation with wide and irregular glycogen vacuoles in hepatocytes (Figure 2).

3.4. Proximate analysis

The moisture, lipid, and ash did not show significant differences among the groups, but a statistical difference was found between protein levels ($P < 0.05$) (Table 5).

4. Discussion

Prebiotics are defined as nondigestible components metabolised by specific bacteria that promote the improvement of health conditions. These bacteria may play an active role in restricting the presence of intestinal pathogens. However, they can contribute to the healthy growth of the host with their positive effect [9,35]. In recent years, the effects of different prebiotic additives on healthy growth in aquaculture have become a focus and many studies have been concentrated on this issue within this framework. In the present study, *C. gariepinus* achieved better growth performances in different prebiotic (MOS, FOS, GOS) applications. In particular, the addition of MOS at 2 g/kg in dietary feed yielded the best results in terms of growth performance. In the Atlantic salmon (*Salmo salar*), dietary supplementation (10 g/kg) with MOS, FOS, and GOS showed no effect on growth performance [36]. Dietary FOS supplementation in the blunt snout bream (*Megalobrama amblycephala*) contributed positively to growth performance in agreement with our results [37]. In another study, it was reported that FOS supplementation could improve antioxidant activities, nonspecific immune response, and growth performance in the Nile Tilapia (*Oreochromis niloticus*) culture [38]. Another study reported that GOS supplementation in feed showed the highest growth performance, but the FOS-supplemented group did not have improved growth [39]. GOS supplementation (5, 10, and 20 g/kg GOS) in the goldfish (*Carassius auratus gibelio*) did not show a significant difference in weight gain compared to the control group [40]. In the zebrafish (*Danio rerio*), different doses of GOS (5, 10, and 20 g/kg) had no effect on growth parameters [41]. Inconsistencies in the results of the studies are most likely due to the doses of the prebiotic used, species differences, and the durations of the study. As stated with previous research data and the present study, MOS, FOS, and GOS supplementations used in aquaculture can be considered to have a positive effect in terms of growth performance.

Physiological and immune conditions in fish can be directly related to haematological parameters [42,43]. Haematological characteristics have been taken into account for a long time in order to specify the alterations of physiological activities of fish [44]. In our study, the experimental groups (MOS, FOS, and GOS) and the control group did not show a significant difference in terms of the concentrations of ESR, RBC, and WBC. Similarly, in the rainbow trout (*Oncorhychus mykiss*), MOS supplementation did not make a difference in terms of haematological parameters (RBC, WBC, ALT, AST, and glucose) compared to the control group [45]. MOS (0.2 g/kg) did not differ on some haematological parameters (WBC, RBC, Hct, Hb, and total serum protein) of the channel catfish (*Ictalurus punctatus*) [46] and also in the giant sturgeon juveniles (*Huso huso*) [17].

In the present study, better small intestine villi lengths were determined in the MOS2 supplementation groups. A significant increase was reported in villi lengths in *Oncorhynchus mykiss* with dietary prebiotic supplementation [41]. The increase in the absorptive surface area of the intestine results in an increased body weight and feed conversion ratio [47]. Our study findings were consistent with this, and the group (MOS2) with the longest villi length showed the best growth level. As supported by the results of this study, prebiotic supplementation increased the feed intake and led to an increased growth performance by improving the intestinal morphology.

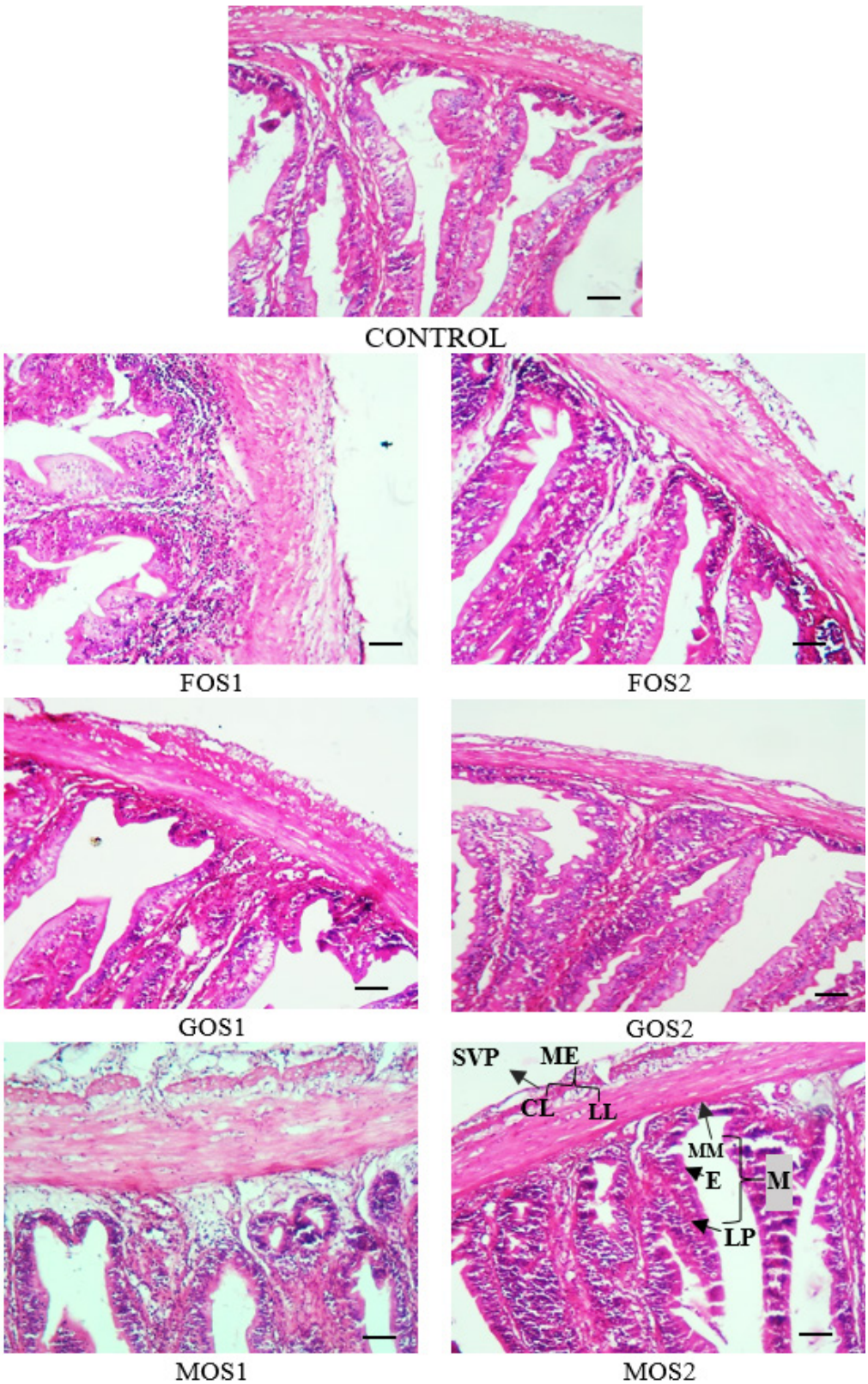

Figure 1. Small intestine sections of *Clarias gariepinus.* SVP: serosa visceral peritoneum, ME: Muscularis externa, CL: Circular layer, LL: Longitudinal layer, M: Mucosa, MM: Muscularis mucosa, E: Epithelia, LP: Lamina propria (Bar 100 μm, H&E, ×10).

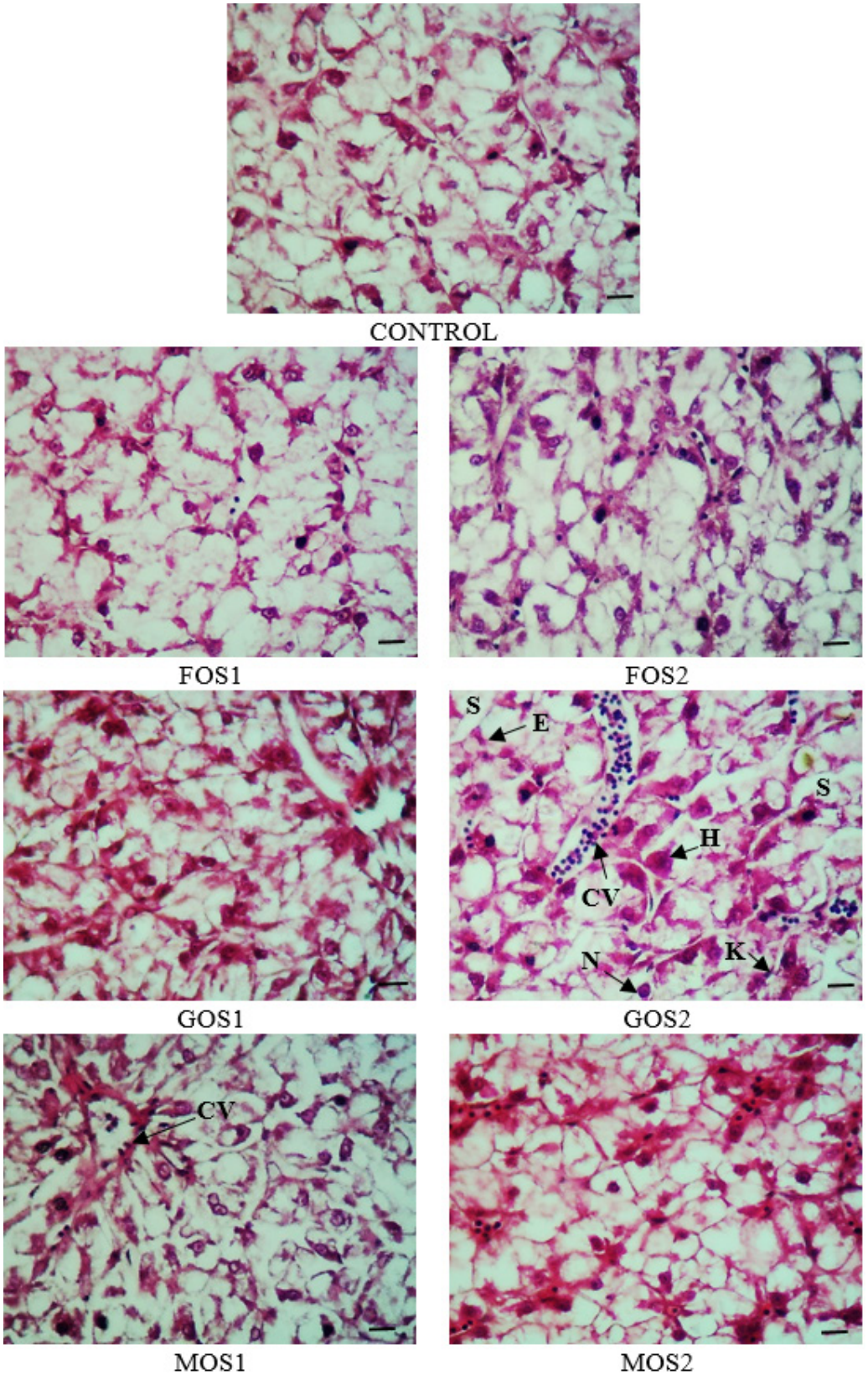

Figure 2. Liver sections of *Clarias gariepinus*. S: Sinusoids, E: Endothelial cell of sinusoid, CV: Capillary vessel, N: Nucleus of hepatocyte, K: Kupffer cell, H: hepatocyte (Bar 50 µm, H&E, ×10).

Table 5. Proximate analysis of *Clarias gariepinus* in control and experimental groups (%).

	Control	FOS1	FOS2	GOS1	GOS2	MOS1	MOS2
Moisture	75.54±0.81[a*]	75.20± 1.03[a]	75.10± 0.39[a]	74.76± 0.59[a]	74.83±0.51[a]	74.77±0.67[a]	74.67±0.93[a]
Protein	16.10±0.50[a]	16.43± 0.22[ab]	16.27± 0.29[ab]	16.99± 0.64[b]	16.33±1.10[ab]	16.90±0.35[b]	16.87±0.35[b]
Lipid	5.93±0.74[a]	5.88± 0.73[a]	5.81± 0.66[a]	5.75± 0.72[a]	5.98± 0.52[a]	5.60±0.87[a]	5.70±0.53[a]
Ash	2.30±0.42[a]	2.46± 0.33[a]	2.35± 0.51[a]	2.50± 0.50[a]	2.47± 0.38[a]	2.49±0.40[a]	2.68±0.24[a]

*The lower-case superscripts indicate differences between the groups ($P < 0.05$).

In the present study, the glucose, ALT, and AST levels were affected by dietary prebiotics. Similar results were observed in *Clarias gariepinus* [48]. However, glucose, AST, and ALT parameters in different fish species did not make a difference between the experimental and control groups [49–51]. These obvious differences can be attributed to the biological characteristics of the fish species and the different prebiotics applied.

In conclusion, dietary MOS (2 g/kg) supplementation can efficiently be used in the diet of the African catfish. The addition of MOS can be calculated as 2 kg per ton of feed for this study outcome. Considering the international average kg price for MOS as 4–5 dollars, this feed additive can be predicted to be economical. The results of this study also suggest that the use of MOS, FOS, and GOS can be considered healthy in terms of haematological and biochemical parameters in *Clarias* diet. Also, the prebiotics tested in the present study have positive effects on increasing the absorptive capacity of the intestine and reduced lipoid vacuolization in the liver tissue.

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Ethical approval

All the applicable international, national, and/or institutional guidelines for the care and use of animals were followed by the authors. The permission of the project for the animal experiment (2017-6-54) was received on 15.03.2017 from the Animal Experiments Local Ethics Committee of Ankara University.

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