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Investigation of the Impact of the Addition of Probiotic Mixtures AQUA CLEAR-S® as Food Supplement on Hematology Parameters and Intestinal Histology of Red Tilapia (*Oreochromis sp.*)

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ABSTRACT

Background: The use of antibiotics in fish feed have been globally restricted due to having its huge negative consequences, dietary prebiotics, probiotics, symbiotics, phytobiotics and other functional dietary supplements are thought to be alternative substitutes. The goal of the study was to determine the effects of adding probiotic mixtures AQUA CLEAR-S® as a feed additive on red tilapia (*Oreochromis sp.*) intestinal histology and hematology parameters.

Methods: The fish were given either the basic experimental nutrition (the control group) or one of three experimental diets that included the basic diet supplemented with 1, 2, or 3 g of AQUA CLEAR-S® for T1, T2 and, T3, respectively. The four experimental treatments were done in three replicates each. Twelve (50 × 40 × 60 cm) aquariums, each with ten fish (avg. wt. 6.00 g±0.17), were used. The studied parameters were basic hematological and immunological blood parameters, antioxidant activities and gut cytology.

Result: Probiotic supplementation significantly improved the hematology parameters in red tilapia fingerlings after probiotic treatments. This trend was continued in the gut architecture. As a result, *Oreochromis sp*. had enhanced health when probiotics were incorporated into their diet. AQUA CLEAR-S®-treated fish also showed a noticeable change in their fish's intestinal histology. The results of the study showed that consuming probiotics increased hematology parameters indicators and gut cytology of red tilapia.

Key words: AQUA CLEAR-S, Hematology parameters, Intestinal histology, Red tilapia.

INTRODUCTION

People have relied on aquaculture and fisheries for sustenance, income and survival for hundreds of millions of years throughout the world (Eissa *et al*., 2022a). Aquaculture has had a significant impact on the world's protein supply in recent decades, contributing to nearly half of all seafood produced globally (Rahman *et al*., 2019; Jahangiri and Esteban, 2018). According to the 2017 fishery yearbook, aquaculture contributes significantly to global food production, with 89 percent of all fishing products used to make personal food (FAO, 2019).

Tilapia are the most widely cultivated species in the world due to their exceptional ability to adapt to a variety of physical and environmental conditions, capacity for in vitro fertilisation, comparative resistance to stressful situations and disease agents, good flesh quality, ability to nourish on a low trophic level and outstanding overall growth on a variety of natural and artificial diets (Lim and Webst). They are the second-most widely cultivated freshwater fish in the world, after carps (Wang and Lu, 2015). However, because tilapia particularly red tilapia (*Oreochromis* sp.) are the species of choice for intensive aquaculture Bauelos-Vargasa *et al*., 2021), they are likely to surpass other cultured fish as the most substantial in the world. Fizzimmons (2006). This is as a result of tilapia's ability to significantly increase fish population without raising operating costs (Rahman *et al*., 2019).

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Aquaculture requires a distribution of fish that is extremely dense in order to prevent diseases brought on by various types of stress during the growing stage. There are consequently more fatalities and monetary losses. To lessen these issues, antibiotics have been used in aquaculture (Nayak, 2010). Hormones, antibiotics and specific salts are some of the most frequently used growth-promoting agents (Klaenhammer and Kullen, 1999). Despite the fact that they aid in growth, improper use of them can cause harm to people, animals and the environment, as well as the emergence of dangerous bacteria that are antibiotic-resistant (Rahman *et al*., 2019). Antibiotics can be replaced with good bacteria known as probiotics to combat harmful bacteria (Camila Sayes *et al*., 2018). Other studies have concentrated on giving probiotic supplements to fish to promote growth (Lara-Flores *et al*., 2003). (Eissa *et al*., in 2022b,c) provided a comprehensive analysis of the potential use of probiotics in aquaculture. The health of the waters, which AQUA CLEAR-S® was made specifically to maintain and improve, determines the lifespan, development and yield quality of fish, prawns and crab. Additionally, it allows aquatic organisms to develop successfully and healthily. The physical-chemical properties of pond water can be stabilised with AQUA CLEAR-S®; it maintains total alkalinity, maintains the ideal pH level and increases D.O. levels in the pond for extended periods of time, allowing fish, prawns and crab to thrive. Remove harmful gases from pond water, such as nitrites, H2S and ammonia. It can work as a sanitizer after removing the dangerous pathogenic bacteria (https://www.flipkart.com/biofit-aqua-clear-250g-aquatic-plant-fertilizer/p/itmfd6dc3 b3a3128). In this study, the haematology parameters and histopathology of red tilapia were all examined in relation to the addition of probiotic combinations known as AQUA CLEAR-S® as feed additives.

MATERIALS AND METHODS

The research was conducted at Hada Al-Sham of Jamoom, Saudi Arabia. A total of 120 red tilapias (*Oreochromis* sp.) fingerlings (avg. wt. 6.00 g±0.17), have been obtained from a private farm and reared equally (10 fingerlings each) in 12 glass aquariums (50 × 40 × 60 cm, 90 L). The fish were acclimated for two weeks and fed a commercial basal diet. The commercial probiotic used in this study was AQUA CLEAR-S where 1 kg of AQUA CLEAR-S comprises, *Bacillus subtilis* 10 × 10^{11} CFU, *Bacillus licheniformis* 7 × 10^{11} CFU, *Lactobacillus acidophilus* 9.8 × 10^{11} CFU, *Saccharomyces cerevisiae* 9 × 10^{11} CFU. The control and experimental diets' feed were formulated according to the nutritional requirement of red tilapia (Table 1).

Aquariums were randomized placed in one of following four treatments (three groups for each therapies).
Feeding fish on a commercial diet without probiotic (T0).
Feeding fish on an enriched diet with 1.0 gm AQUA CLEAR-S per kg diet (T1).
Feeding fish on an enriched diet with 2.0 gm AQUA CLEAR-S per kg diet (T2).
Feeding fish on an enriched diet with 3.0 gm AQUA CLEAR-S per kg diet (T3).

Fish were fed three times daily at 8:00, 12:00 and 16:00 at a rate based on their weight (7% in the first two weeks and 6% in the following two weeks for all treatments) for 56 days. Each experimental group's fish were live-weighed in every two weeks in order to estimate the quantity of food received throughout the trial period. Water quality parameters were monitored daily throughout the feeding study and treatments were assessed in stable aerated water with a daily 25% water change. The water temperature, pH, dissolved oxygen, salinity and ammonia (NH4) were determined using the method of Boyd (2019) and recorded as 26.47±0.03°C, 7.63±0.03, 6.80±0.06 mg/l, 0.50±0.01 ppt and 0.39±0.00 mg/l, respectively.

Three fish from each aquarium were used to collect blood and serum samples via the dorsal blood artery using heparinized tubes as an anticoagulant and without heparin, respectively. The fishes were anesthetic using tricaine methane sulfonate (MS-222) at a dosage of 100 mg/L. Blood was separated at 3500 g for 15 minutes at 4°C to produce the serum, which was then stored at 20°C for other tests.

According to Houston (1990) and Stoskopf (1993), red blood cells (RBC) were determined using a hemocytometer with Natt and Herrick's solutio. After adding Drabkin's solution, the haemoglobin was measured using Blaxhall and Daisley's (1973) method. According to Peters Jr. (1970),

Table 1: Ingredients content and chemical analysis of the tested diet (% on dry matter basis).

	Ingredients composition			
	T0	T1	T2	T3
Fish meal (70% CP)	210	210	210	210
Soybean meal (44% CP)	210	210	210	210
Yellow corn	220	220	220	220
Wheat bran	100	100	100	100
Rice bran	200	199	198	197
Probiotics	0	1	2	3
Linseed oil	20	20	20	20
Vitamins premix (1)	20	20	20	20
Minerals premix (2)	20	20	20	20
	1000	1000	1000	1000
	Chemical analysis (%)			
Crude protein (CP)	30.33	30.28	30.32	30.42
Ether extract (EE)	5.62	5.60	5.61	5.60
Ash	5.61	5.63	5.61	5.60
Crude fiber (CF)	5.31	5.30	5.33	5.35
Nitrogen free extract (NFE)	53.13	53.21	53.12	53.33

Doumas and Biggs (1972) and Peters Jr *et al*. (1982), the total protein, albumin and globulin contents of the collected serum were assessed. According to Reitman and Frankel (1957), the enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were measured. Heinegard and Tiderstrom's (1973) formula was used to calculate creatinine. According to Trinder (1969), serum glucose was measured by a "Boehringer" blood sugar testing protocol.

Three fishes were chosen randomly from each feeding group and kept in 40% ethyl alcohol (Dawood *et al*., 2020) for histological analysis. Neutral buffer formaldehyde liquid was used to fix specimen from the intestines. The tissue specimens were dehydrated in escalating ethanol yield after completed fixation, cleared in xylene and then embedded in paraffin wax. Leica 2025 rotary microtome was used to slice paraffin blocks into 5 m sections, which were then colored with hematoxylin and eosin (H & E) and viewed below a light microscope (Bancroft and Gamble, 2008).

The results were assessed using the one-way analysis of variance (ANOVA) test in SPSS version 26. Variations were considered significant at $P<0.05$. To determine the linear effects of probiotic supplementation on the tested variables, polynomial regression analysis was performed (Yossa and Verdegem, 2015).

RESULTS AND DISCUSSION

The RBCs showed a dramatic elevation ($P<0.05$ in fish which used probiotic (Table 2). The ALT and glucose levels dramatically reduced ($P<0.05$) in T1, T2 and T3 fish groups compared to the control group (T0). Furthermore, the AST and alkaline phosphatase decreased in the various tested groups relative to control. The supplementation of probiotic dramatically elevated the serum total protein and albumin and globulin levels ($P<0.05$) relative to the control group after 56 days. The lowest levels of creatinine were observed in T1, T2 and T3. Hemoglobin (Hb) (g dl-1), Hematocrit (Hct) (%), Mean corpuscular hemoglobin (MCH) (pg) and mean corpuscular hemoglobin concentration (MCHC) (%) increased. While Mean corpuscular volume (MCV) (fl) decreased. Urea and Uric acid decreased in treatment groups relative to the control group. The haemato-biochemical parameters are critical in determining the health of aquatic animals. All of the haemato-biochemical parameters measured in this study on red tilapia were within normal limits, compared to the earlier research done by Rathore *et al*. (2021). Hemato-biochemical parameters are useful for assessing fish health, nutritional status and ability to adjust to their environment (Abdel-Tawwab, 2016; Adeshina *et al*., 2019).

Total protein, albumin and globulin levels were greatest in the probiotics treatments, showing that the probiotics-fed fish were better than the control group. As probiotic levels rose, so did these markers. The results are comparable to those of Kamgar and Ghane (2014) and Nargesi, Falahatkar (2020), however they diverge at lower glucose concentrations. The usage of probiotics, which enhance the intestinal flora and consequently digesting and nutrient absorption, may be responsible for the protein profile (Eshak *et al*., 2010). Fish have a considerable immune response, as evidenced by the rise in total protein, albumin and globulin levels (Al-Dohail *et al*., 2009).

The findings revealed that fish fed probiotics had considerably greater RBC, Hb and Ht levels than the control group. These results demonstrated that Red tilapia without anemia signs had increased hematological parameters due to feeding probiotics. These findings suggest improved fish health since they are associated with increased erythropoiesis and hemosynthesis. According to earlier studies (Mişe Yonar *et al*., 2013; Priyadarsini, 2014; El-

Table 2: Testing biochemical indices (at the end of the experiment) of red tilapia treated with probiotic for 56 days.

Parameters	T0	T1	T2	T3
ALT	47.49±0.91	46.51±0.13	45.83±0.24	45.34±0.25
AST	126.23±2.08	121.63±0.71	118.53±0.51	117.76±0.67
ALP	26.47±0.35	23.68±0.43	21.69±0.04	20.95±0.16
RBCs	1.20±0.02	1.37±0.01	1.44±0.01	1.45±0.01
Hb (g dl-1)	6.16±0.02	7.35±0.07	7.90±0.01	7.93±0.01
Hct (% ((pcv)	27.36±0.12	28.31±0.08	28.71±0.05	28.88±0.01
MCV (fl)	237.88±2.69	212.04±0.62	200.34±1.64	192.09±2.58
MCH (pg)	51.19±0.95	53.75±0.19	54.76±0.41	54.72±0.36
MCHC (%)	23.02±0.53	25.46±0.09	26.77±0.29	27.55±0.31
TP	3.21±0.05	3.41±0.01	3.69±0.05	3.96±0.02
Albumin	1.38±0.03	1.61±0.01	1.48±0.04	1.85±0.03
Globulin	1.83±0.02	1.89±0.00	2.21±0.01	2.10±0.01
Glucose	129.31±0.73	126.07±0.19	126.24±0.00	123.96±0.11
Creatinine	61.70±0.70	56.14±0.87	51.48±0.92	49.13±0.22
Urea	23.48±0.66	21.29±0.31	19.64±0.15	18.67±0.24
Uric acid	1.77±0.04	1.49±0.08	1.14±0.02	1.04±0.04

(Data are expressed as means±S.D).

Barbary, 2018; Enis Yonar *et al*., 2019; Mohamed *et al*., 2020), adding curcumin to the diet enhances hematological markers in a range of fish species. These outcomes provide credence to those studies.

Systematically evaluated markers of the status of the liver are the aminotransferases AST, ALT and ALP (Murray *et al*., 2003; Molina *et al*., 2005). The results show that the fish in the current research weren't under any stress. Fadl *et al*. (2020) and Xu *et al*. (2020) showed the blood AST, ALT and ALP activity of fish fed probiotic-supplemented diets was dramatically reduced. According to Eissa *et al*. (2022c), *P. vannamei* given various dosages of probiotic supplements had a reduction in AST, ALT and ALP levels in contrast to the control.

Different investigators have reported that liver enzymes levels vary depending on the used probiotics used Their levels were dramatically decreased in tilapia plasma after dietary treatment of two probiotic bacteria strains (*Micrococcus luteus* and *Pseudomonas spp*.), which has led to the discovery that their levels differ depending on the probiotics utilized in research (Abd El-Rhman *et al*., 2009). However, in a different research, fish that received dietary supplements including *Lactobacillus plantarum*, *Lactobacillus acidophilus*, or *Saccharomyces cerevisiae* and olive flounder (*Paralichthys olivaceus*), had considerably higher levels of AST, ALT and ALP (Harikrishnan *et al*., 2010).

The findings of the current study concur with those of Hassanien *et al*., (2017) and Kurdomanov *et al*., (2019), who discovered that probiotic-treated diets decreased AST, ALT and ALP levels when as compared to control diets, but they disagree with Al-Hisnawi and Beiwi (2021), who discovered that diets supplemented with *Bacillus subtilis* and Ghiasi, Binaii (2018), who reported that differences between studies might be explained by differences in the probiotics' kind, amount, fish species tested, treatment period and/or external conditions (Eissa *et al*., 2022b).

The findings showed that probiotic supplementation increased mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). The average corpuscular volume decreased (MCV) as well. These findings contradicted those of Knoph and Thorud (1996), who reported a rise in MCV.

In contrast, the probiotic supplemented groups showed a reduction in their levels of creatinine, urea and uric acid. In contrast to Farghaly *et al*., (1973) who showed no change in creatinine, urea and uric acid upon consuming the probiotic reveling its safety for the fish's kidney metabolic function.

Histological results of the intestinal villi of the T2 and T3 groups were branched, markedly lengthened and the apical portion of the T3 group's intestinal villi had moderate sloughing. In the controlled condition, the intestinal villi's apical portion was clearly sloughing (T0) as shown in (Fig 1, 2). Fish growth, illness resistance and feed consumption are all greatly impacted by the growth of the internal organs, particularly the gut. In the current investigation, the histology of the tilapia treated with commercial probiotics revealed noticeably better intestinal developments than the control group. The current study's findings are in line with those of

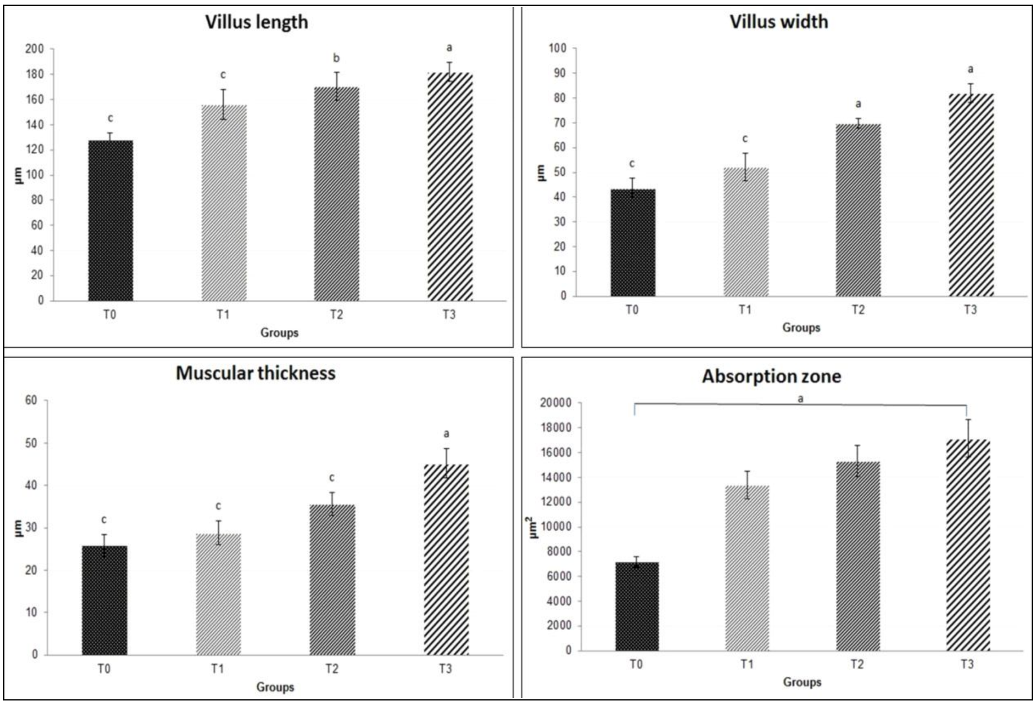
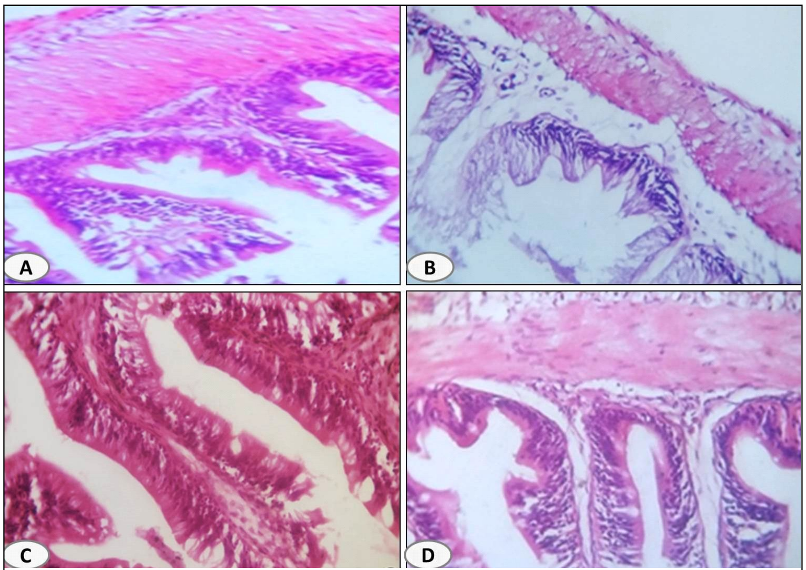


Fig 1: Effect of probiotic diets on intestinal histology (Data are represented as means ± standard deviations) of red tilapia.



(a) Control (T0), (b) T1, (c) T2 and (d) T3
(scale bar = 50 μm). M= muscular layer and V= villi.

Fig 2: Histological sections of red tilapia intestine fed with the experimental diets.

other research in terms of the beneficial effects of probiotics during larval stages and beyond, whether as a feed addition, a water additive, or even as an enrichment to live foods (rotifer, Artemia, *etc*). (Lotfy, 2015; Kuebutornye *et al*., 2019; Eissa *et al*., 2022b).

CONCLUSION

The results of this study exhibited that dietary probiotics improved basic hematological parameters and intestinal architecture of red tilapia. The best performance was observed in 3.0 gm AQUA CLEAR-S per kg fish diet and this dose is suggested therefore.

Conflict of interest: None.

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