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Effects of Dietary Mannan Oligosaccharide and Fructo Oligosaccharide Combinations on the Culture Performance of Red Swamp Crayfish

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ABSTRACT

This research explored the impact of dietary prebiotics on the growth performance of red swamp crayfish over two distinct 90-day trials (each with 3 replicates). In the first trial (initial weight: 0.085 g, 7 experimental groups), mannan-oligosaccharide (M0, M1, M2, M3) and fructo-oligosaccharide (F0, F1, F2, F3) were added at concentrations of 0, 1, 2, and 3 g kg^{-1}. The highest weight gain (WG) and specific growth rates (SGR) were recorded in the M3 group (WG: 8.05 g, SGR: 5.07) and F3 group (WG: 8.00 g, SGR: 5.06). Similarly, the M3 and F3 groups showed the most favorable feed conversion ratios (FCR) and survival rates (SR). In the second trial (initial weight: 0.087 g, 10 experimental groups), the combined use of MOS and FOS (M3+F3) delivered the best performance (WG: 8.82 g, SGR: 5.12, FCR: 1.29, SR: 93%), compared to the M1+F1 group (WG: 6.94 g, SGR: 4.86, FCR: 1.64, SR: 82%). While hepatopancreas tissues remained normal in all groups, the probiotic-supplemented groups exhibited significantly higher crude protein and lower fat content, total hemocyte counts, and intestinal bacteria counts compared to the control group ($p<0.05$). A combination of 3 g kg^{-1} MOS and FOS is recommended to enhance crayfish farming productivity.

Keywords: Growth, Crayfish, *Procambarus clarkii*, prebiotics, proximate analysis.

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Mannan-Oligosakkarit ve Frukto-Oligosakkarit Kombinasyonlarının Kırmızı Bataklık Kereviti Yetiştiriciliği Performansı Üzerine Etkileri

Öz: : Bu araştırma, iki farklı deneme boyunca (her biri 3 tekrarlı ve 90 günlük) kırmızı bataklık kerevitlerinin büyüme performansına yemle verilen prebiyotiklerin etkileri incelemiştir. Birinci denemede (başlangıç ağırlığı: 0,085 g, 7 deney grubu), mannan-oligosakkarit (M0, M1, M2, M3) ve frukto-oligosakkarit (F0, F1, F2, F3) 0, 1, 2 ve 3 g kg^{-1} düzeylerinde eklenmiştir. En yüksek ağırlık artışı (WG) ve spesifik büyüme oranları (SGR) M3 grubunda (WG: 8,05 g, SGR: 5,07) ve F3 grubunda (WG: 8,00 g, SGR: 5,06) kaydedilmiştir. Aynı şekilde, M3 ve F3 grupları en iyi yem değerlendirme oranlarına (FCR) ve hayatta kalma oranlarına (SR) sahip olmuştur. İkinci denemede (başlangıç ağırlığı: 0,087 g, 10 deney grubu), MOS ve FOS'un (M3+F3) birlikte kullanımı, M1+F1 grubuna kıyasla en iyi sonuçları vermiştir (WG: 8,82 g, SGR: 5,12, FCR: 1,29, SR: %93; M1+F1 WG: 6,94 g, SGR: 4,86, FCR: 1,64, SR: %82). Tüm gruplarda hepatopankreas dokuları normal kalırken, probiyotik takviyeli gruplarda ham protein ve yağ seviyeleri anlamlı derecede daha yüksek, toplam hemosit sayısı ve bağırsak bakterileri sayısı ise kontrol grubuna kıyasla daha düşük bulundu ($p<0.05$). *Procambarus clarkii* yetiştiriciliğinin verimini artırmak için 3 g kg^{-1} MOS ve FOS kombinasyonunun kullanılması önerilmektedir.

Anahtar kelimeler: Büyüme, kerevit, *Procambarus clarkii*, prebiyotik, besin bileşen analizi.

How to Cite

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Introduction

The rapid growth of the global population is increasing the demand for quality food sources. Challenges like accessing affordable animal protein and climate change scenarios are among the most discussed topics. Especially during and after the COVID-19 pandemic, disruptions in the supply chain made us realize the importance of local agricultural production and the difficulties of external dependency. Among the self-sufficiency areas of

countries, food production is the most crucial. Aquatic animal products have significant nutritional and economic value. Therefore, aquaculture has the potential to meet human nutritional needs in terms of quantity, quality, and diversity (Genc et al. 2020). However, the aquaculture sector often resorts to using chemicals and antibiotics to reduce disease risks in intensive production. In 2006, the European Union banned the excessive and unnecessary use of antibiotics in animal production (except under veterinary supervision) (Wall et al. 2016). Consequently, restrictions on antibiotic use in aquaculture have also been introduced based on the recommendations of the European Union and FAO (Boix et al. 2014). The ban on chemical agents like antibiotics, which leave residues, has led to increased research into alternative feed additives that can promote healthy growth in animals. In this context, the effectiveness of organic acids, enzymes, probiotics, prebiotics, and synbiotics is being tested (Barug et al. 2006; Akhter et al. 2015; Guerreiro et al. 2018; Reverter et al. 2021; Hoseinifar et al. 2024; Genc et al. 2024a; Genc et al. 2024b). It has been suggested that adding prebiotics to feed can have positive effects on animal health, thereby enhancing efficiency and sustainability in aquaculture (Wee et al. 2022).

Following finfish, decapod crustaceans represent a substantial market demand in the aquaculture industry. Among decapod crustaceans, the red swamp crayfish (*Procambarus clarkii*) ranks as the second most cultivated species, following the Pacific white shrimp (*Penaeus vannamei*). This crayfish species has become a common product that can be cultured in all regions except Antarctica and Oceania (Hobbs Jr 1988; Hobbs Jr et al. 1989; Gherardi 2006; Lodge et al. 2012; Souty-Grosset et al. 2016). The red swamp crayfish is predominantly produced in China and the United States (FAO 2022). Secondary immune system development in decapods is not as advanced as it is in vertebrates, thus requiring constant immune stimulation for healthy culture. Prebiotics are easily accessible and easy-to-apply components that serve as immune stimulants (Cerezuela et al. 2011; Ringø et al. 2010; Dinçer 2022). Prebiotics are fermentable food components resistant to gut enzymes, playing a role in the proliferation and activity of beneficial bacteria in the intestines of terrestrial and aquatic animals. Prebiotics can increase farming efficiency by enhancing nutrient utilization (Gibson and Roberfroid 1995; Manning and Gibson 2004; Guerreiro et al 2014; Ringø et al. 2010, 2014; Akhter et al. 2015).

Among the prebiotics used in aquaculture are β-glucan, inulin, arabinoxylan oligosaccharide (AXOS) (Li et al. 2021), mannan oligosaccharide (MOS) (Mazlum et al. 2011; Genc et al. 2007), galacto-oligosaccharide (GOS), fructo-oligosaccharide (FOS), galacto-glucomannan (GGM), isomalto-oligosaccharide (IMO), and xylo-oligosaccharide (XOS) (Wee et al. 2022). MOS and FOS, in particular, have been reported to improve gut health, boost immune systems, and promote growth (Gibson et al. 2017; Akhter et al. 2015; Assan et al. 2022). MOS is a component derived from the cell wall of the yeast *Saccharomyces cerevisiae*. It is stable at both high and low temperatures, does not react with feed ingredients, and has the ability to suppress mold (Ringø et al. 2010, 2014; Torrecillas et al. 2014; Song et al. 2014; Genc and Kumtepe 2024). Dietary MOS can enhance immune response and prevent pathogen proliferation in the digestive system (Santin et al. 2001; Patterson and Burkholder 2003; Song et al. 2014). FOS, derived from sucrose molecules, is an indigestible compound that has been reported to enhance immune response by increasing the gut bacterial diversity of the host (Dong and Wang 2013). Moreover, there are studies indicating that FOS enhances feed utilization (Ringø et al. 2010; Zhang et al. 2010; Ringø et al. 2014; Song et al. 2014; Guerreiro et al. 2014, 2016).

The cambarid family of freshwater crayfish (*Procambarus* spp.) is native to the Americas, while the astacid family (*Astacus* spp., *Pontastacus* spp.) is found in Europe and Asia, and the parastacid family (*Cherax* spp.) is distributed in Australia and its surroundings (Kumlu 2001; González et al. 2009; Kumlu 2010). Compared to the European crayfish (*Astacus astacus*) and the Eastern European/Turkish crayfish (*Pontastacus leptodactylus*), the red swamp crayfish has higher environmental tolerance and disease resistance. This species is preferred in aquaculture due to its short incubation period (20-25 days) and ability to reproduce multiple times throughout the year (Arslan 2024). The red swamp crayfish is appreciated for its taste in the food industry and its appearance in the aquarium industry (FAO 2009; Holdich 2002). It has been suggested that culturing this species under controlled conditions (in recirculating systems) could reduce the risk of invasion and benefit the food and aquarium sectors (Arslan 2024). However, heterogeneous growth and cannibalism are issues in the culture of *P. clarkii* (Dinçer 2022; Byeon and Lee 2024).

To address these problems, it has been found beneficial to use shelter to prevent cannibalism under controlled conditions (Karplus et al. 1995; Ramalho et al. 2008; González et al. 2011; Huang et al. 2011; Mazlum et al. 2017; Su et al. 2020; Yu et al. 2020). A hypothesis has been developed prioritizing the use of prebiotics to enhance culture efficiency. There are only a limited number of studies addressing the supplementation of MOS and FOS in red swamp

crayfish (*P. clarkii*) culture, with none focusing specifically on the potential effects of their combined use. Therefore, this study aims to determine the effects of different doses of MOS and FOS prebiotics, as well as their combinations, added to species-specific experimental diets on the growth parameters and nutrient composition of red swamp crayfish under controlled conditions (indoor tanks/laboratory scale).

Materials and Methods

Location, broodstock maintenance, and juvenile production

The feeding trials were conducted at the Department of Fisheries and Aquaculture Engineering, Faculty of Agriculture, Ankara University, Ankara, Türkiye. Juvenile red swamp crayfish (*P. clarkii*) were produced by breeding four female and four male broodstock at the research unit. Mating behaviors were observed over a two-week period, during which crayfish were provided a diet of trout pellet feed (45% crude protein), spinach leaves (blanched), and fresh peas. The matured females and males were stocked for mating in an environment equipped with 5-6 cm diameter PVC pipes serving as hiding areas, under clear water conditions with aeration, filtration, and circulation providing a total water volume of 200 L. Successful mating was confirmed by the observation of pleopodal eggs after approximately one month. Crayfish carrying pleopodal eggs were transferred to an incubation tank maintained at 24ºC, where fry hatching occurred after about 22 days. When juvenile crayfish started to detach from the pleopods and move independently on the tank floor, approximately 7 days after hatching, the females were removed from the tank. The juveniles were fed for 10 days with an experimental diet prepared in ground form, in accordance with the recommended formulation for crustaceans (described in the following section: *Experimental Diets and Feeding*). After this period, the juveniles were weighed and distributed into experimental tanks. Their acclimation to the experimental tanks was monitored for three days before the trials commenced. Details of the experimental plans (Trial I and Trial II) are presented below.

Experimental diets and feeding

An experimental diet formulation was prepared following the recommendations for crustaceans by NRC (2011). The raw materials were ground, and MOS and FOS prebiotics were added at 0, 1, 2, and 3 g kg^{-1}. The mixture was then pelletized (using a cold pelleting machine, Pasfil, Istanbul) into pellets 1.2 mm in diameter and 3-4 mm in length. The pellets were conditioned with steam under 1 ATM pressure for approximately 20 minutes to achieve gelatinization. After drying in an oven at 40ºC for 24 hours, the pellets were ground and broken (in suitable sizes for crayfish juveniles), labeled and stored at -18ºC. The basal diet had a nutritional composition of 90.50% dry matter, 6.05% ash, 38.14% protein, and 9.13% lipid (Table 1, Table 2). During the trials, feeding was conducted three times daily according to a graduated schedule. Specifically, the feeding rates were: 8% of live weight for days 0-15, 7.2% for days 15-30, 6.6% for days 30-45, 5.8% for days 45-60, 5% for days 60-75, and 4.2% from day 75 to day 90. Feed amounts were determined based on bi-weekly live weight measurements (Croll and Watts 2004; Dinçer 2022).

Table 1. The composition of experimental diets for Trial I (per 100 g)

	K	1M	2M	3M	1F	2F	3F
Fish meal[a]	30	29.8	29.8	29.8	29.8	29.8	29.8
Soy pulp meal[b]	12	11.8	11.8	11.8	11.8	11.8	11.8
Corn gluten[c]	13	12.8	12.8	12.8	12.8	12.8	12.8
Wheat flour[d]	36.3	35.9	34.9	33.9	35.9	34.9	33.9
Fish oil[e]	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Soy lecithin[f]	2	2	2	2	2	2	2
Vitamin premix[g]	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Mineral premix[g]	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin C[g]	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Guar gum + Cholestrol[h]	3	3	3	3	3	3	3
MOS	**0**	**0.1**	**0.2**	**0.3**	**0**	**0**	**0**
FOS	**0**	**0**	**0**	**0**	**0.1**	**0.2**	**0.3**

table continues

Proximate analysis (%)							
Dry matter	90.50	90.59	90.59	90.64	90.59	90.59	90.64
Crude ash	6.05	6.06	6.08	6.08	6.06	6.06	6.08
Crude protein	38.14	38.07	38.03	38.01	38.07	38.03	38.01
Crude lipid	9.13	9.06	9.06	9.06	9.06	9.06	9.06

[a]Anchovy meal, Sürsan Aquculture Ltd. Samsun, Turkiye, [b]Kırcı Soy Producs, Balıkesir, Türkiye, [c]Cargill, İstanbul, Türkiye, [d]İpek Wheat Fab., Nevsehir, Türkiye, [e]Anchovy oil, Sürsan Aquculture Ltd. Samsun, Türkiye, [f]Sigma Aldrich Chemicals, St. Louis, MO, ABD, [g]DSM Food producs, Türkiye, [h]Guar gum, Kartal Chemical Products Ltd, Istanbul, Turkiye, Cholesterol, Sigma, Germany.

Table 2. The composition of experimental diets for Trial II (per 100 g)

	K	**1M1F**	**1M2F**	**1M3F**	**2M1F**	**2M2F**	**2M3F**	**3M1F**	**3M2F**	**3M3F**
Fish meal[a]	30	29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8	29.8
Soy pulp meal[b]	12	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8	11.8
Corn gluten[c]	13	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8	12.8
Wheat flour[d]	36.3	34.9	33.9	32.9	33.9	32.9	31.9	32.9	31.9	30.9
Fish oil[e]	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Soy lecithin[f]	2	2	2	2	2	2	2	2	2	2
Vitamin premix[g]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Mineral premix[g]	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Vitamin C[g]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Guar gum + Cholestrol[h]	3	3	3	3	3	3	3	3	3	3
MOS	**0**	**0.1**	**0.2**	**0.3**	**0**	**0**	**0**	**0**	**0.1**	**0.2**
FOS	**0**	**0**	**0**	**0**	**0.1**	**0.2**	**0.3**	**0**	**0**	**0**
Proximate analysis (%)										
Dry matter	90.50	91.15	91.15	91.15	91.37	91.37	91.37	91.47	91.47	91.59
Crude ash	6.05	6.08	6.08	6.08	6.17	6.11	6.11	6.19	6.19	6.19
Crude protein	38.14	37.71	37.71	37.71	37.62	37.62	37.62	37.55	37.55	37.55
Crude lipid	9.13	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.06	9.04

[a]Anchovy meal, Sürsan Aquculture Ltd. Samsun, Turkiye, [b]Kırcı Soy Producs, Balıkesir, Türkiye, [c]Cargill, İstanbul, Türkiye, [d]İpek Wheat Fab., Nevsehir, Türkiye, [e]Anchovy oil, Sürsan Aquculture Ltd. Samsun, Türkiye, [f]Sigma Aldrich Chemicals, St. Louis, MO, ABD, [g]DSM Food producs, Türkiye, [h]Guar gum, Kartal Chemical Products Ltd, Istanbul, Turkiye, Cholesterol, Sigma, Germany.

Trial I: Effects of different levels of dietary MOS and FOS on the culture performance of red swamp crayfish

The feeding trial was conducted over 90 days using feeds supplemented with different levels of MOS and FOS prebiotics. The experiment was carried out under controlled conditions with a daily water exchange rate of 3%, aeration, and a photoperiod of 12 hours light and 12 hours dark in tanks with a water volume of 40 L. Each tank housed 15 crayfish, corresponding to a density of 15 crayfish per 0.24 m^2. A total of seven groups, including a control group, were set up with three replicates each. Juvenile crayfish with an initial weight ranging from 0.084 to 0.085 g were randomly selected and distributed according to a completely randomized design. To simulate natural conditions, complex nets and plastic pipes were placed in the tanks as hiding areas. During the experimental period, feeding was performed three times daily following the schedule outlined in the feeding plan (starting at 8% of live weight for the first 15 days and gradually decreasing to 4.2% for the final 15-day period). Water quality parameters, including dissolved oxygen, pH, water temperature, and ORP (oxidation-reduction potential), were measured daily, while nitrite, nitrate, total ammonia nitrogen, and total phosphorus levels were measured weekly. At the end of Trial I, the effectiveness of prebiotic feed additives was evaluated based on growth parameters and nutrient composition analysis.

Trial II: Effects of dietary MOS and FOS combinations on the culture performance of red swamp crayfish

In Trial II, the prebiotic doses used in Trial I were applied in combination to investigate their effects. Nine different prebiotic combinations were formed by using one dose of MOS and one dose of FOS, as applied in the first trial. The second trial comprised ten groups, including a control group, with juvenile red swamp crayfish distributed into 30 tanks following a completely randomized design, with three replicates per group (40 L tanks, 15 crayfish per 0.24 m^2). Juvenile crayfish with an initial weight of 0.087-0.089 g were used at the beginning of Trial II. Feeding was conducted three times a day, starting with 8% of the body weight for the first 15-day period and decreasing to 4.2% for the last 15 days, under the same conditions as Trial I (3% daily water exchange, aeration, and a 12-hour light/12-hour dark photoperiod). Water quality parameters, including dissolved oxygen, pH, water temperature, and ORP, were measured daily, while nitrogenous compounds and total phosphorus were measured weekly. At the end of Trial II, the prebiotic combinations that showed the best culture performance in crayfish were determined.

Water quality parameters

Water quality parameters such as temperature, dissolved oxygen ad pH were daily monitored (YSI Pro20 and YSI EcoSense) in tanks. Additionally, nitrite, nitrate, total ammonia and phosphate were weekly determined using the photometric kit method (Hanna, HI801-01 iris Visible Spectrophotometer, USA) according to the protocol. Moreover, Oxidation Reduction Potential (ORP) measurement (Pinpoint ORP Monitor, American Marine Inc.) was conducted as an additional parameter indicating the suitability of the aquaculture environment for living organisms.

Determination of growth parameters

Body weight measurements were taken at the beginning and 15-day intervals until the end of the experiment'. The formulas of variables including growth and nutrient utilization performance (survival rate (SR), body weight gain (BWG), specific growth rate (SGR), and feed conversion ratio (FCR)) were provided below.

SR (%) = (Day 0 number of crayfish– Final day number of crayfish x 100) / Day 0 number of crayfish

BWG (g) = Final day body weight – Day 0 body weight

SGR (% day^{-1}) = 100 x (ln Final day body weight – ln Day 0 body weight) / Trial period (days)

FCR = Total feed consumption during the trial / Live weight gain

Proximate analysis

After the trials, crayfish samples underwent nutrient analysis following the AOAC protocol (Horwitz 2000). Initially, crayfish samples were dried in an oven at 105.0 ± 0.5ºC until their weights stabilized. Subsequently, protein content was determined using the Kjeldahl method on whole-body dry matter. The crude lipid level was calculated using the Soxhlet extraction protocol. Lastly, ash content was determined using the incinerator protocol (8 hours, 525ºC). The following formulas were employed for the calculations:

Humidity (%) = (dry sample weight - wet sample weight) / (wet sample weight) x 100

Crude Protein (%) = [(titration amount - blank sample x 0,1 x 14,007 x 6,25) / sample amount] x 100

Raw Oil (%) = (the amount of oil in the soxhlet jug/sample amount) x 100

Crude Ash (%) = [(weight of first porcelain cup - weight of last porcelain cup) / sample amount] x 100

Hepatopancreas histomorphology

Two crayfish from each experimental group were sedated in ice water to take a hepatopancreas sample. Subsequently, 0.5 g of hepatopancreas tissue was extracted from the posterior-lateral region of the carapace. The extracted tissue was fixed in a 10 mL buffered formaldehyde solution, placed in histological follow-up cassettes, and labeled. Following a two-day fixation process, the hepatopancreas tissue samples underwent hydration (distilled water), dehydration (increasing ethyl alcohol series), clearing (xylene cold-hot), and paraffinization (warm liquid paraffin series) stages for histomorphological examination using a standard manual protocol. The samples were then transferred to paraffin blocks in tissue embedding containers and sectioned at a 5-6 µm thickness using a rotary microtome (Shandon). The tissue sections were spread into a container with water at 45ºC, transferred to labelled slides, and stained with hematoxylin and eosin following deparaffinization, dehydration, and hydration stages. The stained preparations were examined under a light microscope (CM40 Leica) with a camera, and microphotographs were recorded (Vogt et al. 1985; Genc et al. 2007; Kaya et al. 2019).

Total bacterial counts

Approximately 0.3 g of intestinal contents were collected under aseptic conditions from two crayfish in each tank to determine the total bacterial count in the digestive tract. The samples were transferred into sterile tubes with slight dorsoventral pressure from the anal pore. Dilutions were prepared by placing the samples in tubes containing sterile physiological saline (NaCl 0.85%: 9 mL for ~1 g sample) and serially diluted in the range of 10-1 to 10-7. Inoculation was performed using the patch method on agar plates (Difco^{TM}) in triplicate, representing each group. Petri dishes were incubated at 36 ± 1ºC for 48 hours to calculate the total aerobic bacterial count (TBC) (Okpala et al. 2014). Bacterial count results (average of three plates) are expressed as colony-forming units (CFU g^{-1}).

Total hemocyte count (THC)

Hemolymph samples from red swamp crayfish were collected from the sinus using an anticoagulant syringe (3 mL, BDMicroFine, anticoagulant: 26 mM $C_6H_8O_7$, 100 mM glucose, 450 mM NaCl, 30 mM $C_6H_5Na_3O_7$, 10 mM $C_{10}H_{16}N_2O_8$, pH 4.6). The total hemocyte count was determined using a counting chamber (Neubauer slide) under the microscope (Söderhäll and Smith 1983).

Statistical analysis

Data obtained from the trials were evaluated using a one-way analysis of variance (ANOVA) and Duncan's comparison test (mean ± standard deviation) after checking the normality and homogeneity of variance. The alpha significance level was set at 0.05. Analyses were conducted using the SPSS program (SPSS 17.0, Chicago, IL, USA).

Results

Water quality assessments

During Trial I, the dissolved oxygen level ranged from 6.46 to 6.53 mg/L, pH varied between 6.59 and 6.74, water temperature ranged from 20.91 to 20.97ºC, and ORP fluctuated between 117 and 153 mV. Weekly assessments revealed nitrite levels between 0.16 and 0.21 mg L-1, nitrate levels between 0.95 and 1.00 mg L-1, total ammonia levels between 0.49 and 0.69 mg L-1 and total phosphate levels between 1.17 and 1.20 mg L-1. Statistical analysis indicated no significant difference in most measured water quality parameters ($p>0.05$), except for ORP, and total ammonia values, which exhibited a statistical difference ($p<0.05$), (Table 3).

In Trial II, dissolved oxygen levels were observed between 5.94 and 6.10 mg L-1, pH levels ranged from 6.25 to 6.32, water temperature fluctuated between 20.9 and 21.0ºC, and ORP ranged from 159 to 165 mV. Weekly measurements indicated nitrite levels between 0.14 and 0.16 mg L-1, nitrate levels between 1.27 and 1.52 mg L-1, total ammonia levels between 0.85 and 0.97 mg L-1, and phosphate levels between 1.17 and 1.46 mg L-1. Statistical analysis did not reveal any significant differences between the groupsin terms of most water quality parameters ($p>0.05$). However, significant differences wereobserved in ORP and phosphate values ($p<0.05$). While no statistical difference was foundbetween the control group and the 2M3F group regarding water phosphate levels ($p>0.05$), a significant difference was noted when compared with the 1M3F, 2M1F, 3M2F, and 3M3F groups ($p<0.05$), (Table 3).

Table 3. Water quality parameters measured during the trials

	Groups	pH*	DO (mg/L)	T (°C)	$NO2^-$ (mg/L)	$NO3^-$ (mg/L)	TAN (mg/L)	T-P (mg/L)	ORP (mV)
Trial I	**Control$_1$**	6,74±0,21^a	6,51±0,05^a	20,95±0,02^a	0,21±0,03^a	1,00±0,12^a	0,57±0,07^{ab}	1,17±0,04^a	123±10^{ab}
	1M	6,65±0,19^a	6,51±0,05^a	20,91±0,03^a	0,19±0,04^a	0,98±0,05^a	0,58±0,09^{ab}	1,18±0,07^a	139±13^{bc}
	2M	6,60±0,25^a	6,46±0,04^a	20,96±0,08^a	0,16±0,05^a	0,95±0,10^a	0,49±0,04^a	1,19±0,08^a	146±19^c
	3M	6,59±0,27^a	6,54±0,04^a	20,93±0,06^a	0,20±0,03^a	0,98±0,18^a	0,69±0,13^b	1,18±0,06^a	117±9,0^a
	1F	6,63±0,15^a	6,53±0,02^a	20,92±0,09^a	0,16±0,04^a	0,98±0,08^a	0,54±0,09^{ab}	1,19±0,05^a	147±9,0^c
	2F	6,66±0,28^a	6,49±0,08^a	20,97±0,01^a	0,20±0,03^a	0,95±0,14^a	0,56±0,07^{ab}	1,19±0,08^a	146±5,0^c
	3F	6,60±0,19^a	6,50±0,09^a	20,93±0,06^a	0,16±0,04^a	0,99±0,12^a	0,61±0,04^{ab}	1,18±0,06^a	153±13^c
Trial II	**Control$_2$**	6,28±0,01^a	6,05±0,52^a	21,0±0,05^a	0,16±0,05^a	1,33±0,21^a	0,93±0,06^a	1,18±0,11^a	162±2,6^{abc}
	1M1F	6,32±0,07^a	6,08±0,49^a	20,9±0,03^a	0,15±0,03^a	1,32±0,24^a	0,85±0,08^a	1,29±0,10^{abc}	159±1,00^a
	1M2F	6,28±0,06^a	6,05±0,55^a	21,0±0,08^a	0,15±0,03^a	1,46±0,18^a	0,86±0,09^a	1,32±0,10^{abc}	165±1,5^c
	1M3F	6,31±0,05^a	5,94±0,50^a	21,0±0,11^a	0,15±0,03^a	1,47±0,15^a	0,91±0,08^a	1,37±0,04^{bc}	163±2,1^{abc}
	2M1F	6,27±0,03^a	6,01±0,49^a	21,0±0,15^a	0,15±0,04^a	1,30±0,40^a	0,86±0,07^a	1,38±0,07^{bc}	162±1,7^{abc}
	2M2F	6,26±0,02^a	6,10±0,50^a	21,0±0,02^a	0,14±0,04^a	1,52±0,14^a	0,92±0,02^a	1,33±0,03^{abc}	163±2,1^{bc}
	2M3F	6,26±0,05^a	5,99±0,52^a	20,9±0,06^a	0,15±0,04^a	1,49±0,08^a	0,90±0,06^a	1,17±0,10^a	164±1,5^{bc}
	3M1F	6,29±0,07^a	6,09±0,52^a	20,9±0,17^a	0,16±0,04^a	1,27±0,36^a	0,91±0,06^a	1,26±0,14^{ab}	160±1,0^{ab}
	3M2F	6,32±0,05^a	6,04±0,49^a	21,0±0,10^a	0,16±0,04^a	1,35±0,22^a	0,92±0,03^a	1,46±0,05^c	164±2,5^{bc}
	3M3F	6,25±0,06^a	6,02±0,47^a	20,9±0,13^a	0,16±0,04^a	1,45±0,22^a	0,94±0,03^a	1,39±0,07^{bc}	165±3,1^c

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS, DO: Dissolved oxygen, T: Water temperature, Nitrite: NO_2^-, Nitrate: NO_3^-, TAN: Total ammonia nitrogen ($NH_3 + NH_4^+$), T-P: Total phosphorus, ORUP: Oxidative reduction potential (mV). Data are presented as mean ± standard deviation.

Growth

In Trial I, MOS and FOS prebiotics were added separately to the feeds as feed additives and fed and given to 7 groups, including the control group, for 90 days. In Trial II, co-administrations of MOS and FOS prebiotics were tested 10 dietary groups for 90 days. The SGR in Trial I reveals a clear trend of improvement with increasing levels of MOS and FOS, reaching the highest in the 3M group (5.07 ± 0.04% day^{-1}). The weight gain in Trial I also followed a similar pattern, with the 3M group exhibiting the highest value (8.05 ± 0.22 g). The FCR results in Trial I support the growth metrics, with the 3M group displaying the best ratio (1.28 ± 0.04 g). Lower FCR values indicate more efficient feed utilization, emphasizing the positive effect of higher MOS levels (Table 4).

In Trial II, the SGR values verified the improvement with 3M3F group showing the highest SGR (5.12 ± 0.03% day^{-1}). This suggests that the combination of MOS and FOS in Trial II synergizes with crayfish growth. The weight gain results in Trial II support the SGR findings, with the best value in 3M3F group achieving the highest value (8.82 ± 0.27 g). The FCR results in Trial II align with the growth metrics, and the 3M3F group exhibits the lowest FCR (1.29 ± 0.04). This underscores the efficiency of feed conversion when using combined prebiotics (Table 4).

Proximate analysis

In Trial I, the comprehensive assessment of whole-body nutrient components resulted (in dry matter basis) from feeding red swamp crayfish with prebiotic-added feeds at varying rates. Notably, the 3M group exhibited the highest crude protein level at 40.07 ± 0.25%. Conversely, the control group displayed the lowest crude protein level, registering at 37.73 ± 0.15% ($p<0.05$). No statistical differences were observed among the Trial I groups ($p>0.05$). Findings from the whole-body nutrientcomponent analysis in Trial II revealed that, when utilizing prebiotic combinations as feedadditives, the 3M2F group exhibited the highest protein content, recording 41.23 ± 0.74%. In contrast, the control group presented the lowestvalue at 38.33 ± 1.02% ($p<0.05$). Regarding ash content, the control group recorded thehighest rate (31.23% ± 1.06%), whereas the 3M2F group maintained the lowest level at 28.53 ± 0.87% ($p < 0.05$). All experimental groups had a similar dry matter value ($p>0.05$). The highest crude lipid content was in the control group (7.50%), and the lowest was in the 3M group (6.30%), (Table 5).

In Trial II, the 3M2F group had the highest crude protein content (41.23%), while the control group had the lowest (38.33%). The control group had the highest crude lipid content (7.37%), and the 3M2F group had the lowest (5.97%).

Table 4. Effects of different dietary MOS, FOS and combinations on growth of red swamp crayfish (90 days)

	Groups	IW (g)*	FW (g)	WG (g)	SGR	FCR	SR
Trial I	**Control$_1$**	0.084 ± 0.001^{a}	7.128 ± 0.505^{a}	7.04 ± 0.51^{a}	4.93 ± 0.08^{a}	1.47 ± 0.10^{b}	84.45 ± 3.85^{ab}
	1M	0.085 ± 0.001^{a}	7.147 ± 0.196^{a}	7.06 ± 0.20^{a}	4.93 ± 0.03^{a}	1.45 ± 0.04^{b}	82.22 ± 3.85^{a}
	2M	0.085 ± 0.001^{a}	7.287 ± 0.446^{a}	7.20 ± 0.45^{a}	4.95 ± 0.07^{ab}	1.43 ± 0.09^{b}	88.89 ± 3.85^{abc}
	3M	0.085 ± 0.001^{a}	8.139 ± 0.219^{b}	8.05 ± 0.22^{b}	5.07 ± 0.04^{b}	1.28 ± 0.04^{a}	93.33 ± 0.00^{c}
	1F	0.085 ± 0.000^{a}	7.157 ± 0.505^{a}	7.07 ± 0.51^{a}	4.92 ± 0.08^{a}	1.46 ± 0.10^{b}	86.67 ± 6.67^{abc}
	2F	0.085 ± 0.000^{a}	7.243 ± 0.241^{a}	7.16 ± 0.24^{a}	4.94 ± 0.04^{a}	1.44 ± 0.05^{b}	86.67 ± 0.00^{abc}
	3F	0.085 ± 0.001^{a}	8.084 ± 0.612^{b}	8.00 ± 0.61^{b}	5.06 ± 0.09^{b}	1.29 ± 0.10^{a}	91.11 ± 3.85^{b}
Trial II	**Control$_2$**	0.087 ± 0.001^{ab}	7.039 ± 0.098^{a}	6.95 ± 0.98^{a}	4.87 ± 0.02^{a}	1.64 ± 0.02^{b}	86.67 ± 0.00^{ab}
	1M1F	0.088 ± 0.001^{ab}	7.031 ± 0.114^{a}	6.94 ± 0.11^{a}	4.86 ± 0.03^{a}	1.64 ± 0.03^{b}	82.22 ± 3.85^{a}
	1M2F	0.087 ± 0.001^{a}	7.123 ± 0.146^{a}	7.04 ± 0.15^{a}	4.89 ± 0.03^{a}	1.62 ± 0.04^{b}	88.89 ± 3.85^{ab}
	1M3F	0.088 ± 0.001^{ab}	7.111 ± 0.438^{a}	7.02 ± 0.44^{a}	4.88 ± 0.06^{a}	1.62 ± 0.09^{b}	91.11 ± 3.85^{ab}
	2M1F	0.088 ± 0.001^{ab}	7.194 ± 0.379^{a}	7.11 ± 0.38^{a}	4.89 ± 0.05^{a}	1.60 ± 0.09^{b}	86.67 ± 6.67^{ab}
	2M2F	0.087 ± 0.000^{a}	7.200 ± 0.043^{a}	7.11 ± 0.04^{a}	4.91 ± 0.01^{a}	1.60 ± 0.01^{b}	91.11 ± 7.70^{ab}
	2M3F	0.088 ± 0.002^{ab}	8.343 ± 0.263^{b}	8.25 ± 0.26^{b}	5.06 ± 0.03^{b}	1.38 ± 0.05^{a}	84.45 ± 3.85^{ab}
	3M1F	0.088 ± 0.001^{ab}	8.548 ± 0.300^{bc}	8.46 ± 0.30^{bc}	5.08 ± 0.03^{bc}	1.35 ± 0.05^{a}	91.11 ± 3.85^{ab}
	3M2F	0.087 ± 0.002^{a}	8.809 ± 0.339^{bc}	8.72 ± 0.34^{bc}	5.13 ± 0.02^{c}	1.31 ± 0.05^{a}	91.11 ± 7.70^{ab}
	3M3F	0.089 ± 0.001^{b}	8.905 ± 0.267^{c}	8.82 ± 0.27^{c}	5.12 ± 0.03^{c}	1.29 ± 0.04^{a}	93.33 ± 0.00^{b}

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS, IW (g): Initial weight, FW (g): Final live weight, BWG (g): Weight gain, SGR (%day^{-1}): specific growth rate, FCR: Feed conversion ratio, SR: Survival rate. Data are presented as mean ± standard deviation.

The control group had the highest crude ash content (31.23%), and the 3M2F group had the lowest (28.53%). There was no significant difference in dry matter content among the groups (Table 5).

Table 5. Effects of different dietary MOS, FOS and combinations on proximate analysis (dry matter basis) of red swamp crayfish (90 days)

	Groups	Crude protein	Crude lipid	Crude ash	Dry matter
Trial I	**Control$_1$**	37.73 ± 0.21^{a}	7.50 ± 0.20^{c}	32.77 ± 0.83^{a}	22.43 ± 0.90^{a}
	1M	38.50 ± 0.52^{ab}	6.9 ± 0.3^{abc}	32.03 ± 1.02^{a}	22.93 ± 0.47^{a}
	2M	39.10 ± 0.62^{bc}	6.8 ± 0.1^{ab}	31.60 ± 1.20^{a}	23.23 ± 0.50^{a}
	3M	40.07 ± 0.25^{c}	6.3 ± 0.4^{a}	31.03 ± 0.38^{a}	23.10 ± 0.85^{a}
	1F	38.07 ± 0.65^{ab}	7.3 ± 0.5^{bc}	31.93 ± 1.02^{a}	23.27 ± 0.55^{a}
	2F	38.33 ± 0.71^{ab}	7.4 ± 0.5^{bc}	32.17 ± 1.35^{a}	22.83 ± 0.95^{a}
	3F	38.03 ± 0.81^{ab}	7.1 ± 0.4^{bc}	32.00 ± 1.23^{a}	23.27 ± 0.60^{a}
Trial II	**Control$_2$**	38.33 ± 1.02^{a}	7.37 ± 0.12^{c}	31.23 ± 1.06^{c}	23.53 ± 1.21^{a}
	1M1F	39.10 ± 1.37^{a}	7.10 ± 0.20^{c}	30.20 ± 0.26^{bc}	23.93 ± 0.67^{a}
	1M2F	39.30 ± 1.04^{ab}	7.13 ± 0.49^{c}	30.03 ± 0.67^{bc}	24.27 ± 1.42^{a}
	1M3F	38.73 ± 1.53^{a}	6.87 ± 0.06^{bc}	30.33 ± 0.64^{bc}	24.63 ± 0.96^{a}
	2M1F	38.87 ± 0.06^{a}	7.30 ± 0.46^{c}	30.27 ± 0.40^{bc}	24.07 ± 0.72^{a}
	2M2F	40.30 ± 0.95^{ab}	6.17 ± 0.32^{ab}	29.63 ± 0.74^{ab}	24.43 ± 1.18^{a}
	2M3F	39.27 ± 0.40^{ab}	6.73 ± 0.60^{bc}	30.20 ± 0.98^{bc}	24.23 ± 0.74^{a}
	3M1F	39.43 ± 1.46^{ab}	6.83 ± 0.12^{bc}	29.87 ± 0.38^{b}	24.30 ± 0.66^{a}
	3M2F	41.23 ± 0.74^{b}	5.97 ± 0.81^{a}	28.53 ± 0.87^{a}	24.77 ± 1.01^{a}
	(table continues…)				
	3M3F	39.03 ± 1.31^{a}	6.87 ± 0.40^{bc}	29.80 ± 0.26^{b}	24.87 ± 0.95^{a}

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS. Data are presented as mean ± standard deviation.

Hepatopancreas histomorphology

In trial I, there were no significant differences in hepatopancreatic tissues among the groups, indicating that the prebiotic supplements did not adversely affect tissue morphology. The star-shaped tubular structure and cell types within appeared normal, affirming the health of the hepatopancreatic tissues. The tubular epithelial morphology confirmed that the prebiotics did not harm the crayfish. In trial II, applying different prebiotic combinations at varying doses did not alter the hepatopancreatic tissue morphology compared to the control group. In summary, Trial II demonstrated that the crayfish from all treatment groups exhibited normal hepatopancreatic tubular tissues, epithelial vacuoles, and tubular digestive and absorption functions without deviation from the expected normal.

Total bacterial counts

Trial I, the 2F group showed the highest bacterial count in the intestinal content ($4.60 \pm 0.26 \times 10^5$ CFU g^{-1}), while the control group had the lowest count ($3.88 \pm 0.13 \times 10^5$ CFU g^{-1}) ($p<0.05$). The 2F group was similar to the 2M ($4.55 \pm 0.40 \times 10^5$ CFU g^{-1}) and 3M groups ($4.43 \pm 0.29 \times 10^5$ CFU g^{-1}) ($p>0.05$). In trial II, the 3M2F group exhibited the highest bacterial count in intestinal content ($5.03 \pm 0.51 \times 10^5$ CFU g^{-1}), whereas the control group had the lowest count ($4.05 \pm 0.21 \times 10^5$ CFU g^{-1}), (Table 6).

Hemolymph parameters

In trial I, the 3M group exhibited the highest total hemocyte count ($81.67 \pm 2.75 \times 10^6$ cell mL^{-1}), while the control group had the lowest count ($38.17 \pm 2.75 \times 10^6$ cell mL^{-1}) ($p < 0.05$). The results indicated a significant increase in the number of hemocytes containing immune cells in crayfish fed with FOS and MOS individually. In trial II, the 3M2F group showed the highest total hemocyte count ($92.00 \pm 5.57 \times 10^6$ cell mL^{-1}), with the control group having the lowest count ($48.33 \pm 1.61 \times 10^6$ cell mL^{-1}). The prebiotic combination supplemented feeds significantly increased the number of hemocytes containing immune cells compared to the control groups ($p < 0.05$), (Table 6).

Table 6. Effects of different dietary MOS, FOS and combinations on total number of aerobic bacteria from intestine and hemocyte counts from hemolymph of red swamp crayfish

	Groups	TAB ($x10^5$ CFU g^{-1})	THC ($x10^6$ cell mL^{-1})
Trial I	**$Control_1$**	$3.88 \pm 0.13^{a*}$	38.17 ± 2.75^{a}
	1M	4.23 ± 0.24^{ab}	61.33 ± 1.04^{c}
	2M	4.55 ± 0.40^{b}	69.17 ± 1.53^{d}
	3M	4.43 ± 0.29^{b}	81.67 ± 2.75^{e}
	1F	4.00 ± 0.14^{a}	51.33 ± 2.75^{b}
	2F	4.60 ± 0.26^{b}	61.00 ± 0.50^{c}
	3F	4.28 ± 0.29^{ab}	57.67 ± 2.52^{c}
Trial II	**$Control_2$**	4.05 ± 0.21^{a}	48.33 ± 1.61^{a}
	1M1F	4.58 ± 0.70^{ab}	58.83 ± 7.01^{b}
	1M2F	4.50 ± 0.42^{ab}	65.17 ± 2.25^{bc}
	1M3F	4.68 ± 0.52^{ab}	68.50 ± 2.65^{cd}
	2M1F	4.83 ± 0.73^{ab}	77.50 ± 2.29^{ef}
	2M2F	4.85 ± 0.72^{ab}	79.00 ± 4.77^{ef}
	2M3F	4.90 ± 0.43^{ab}	74.67 ± 4.54^{de}
	3M1F	4.80 ± 0.34^{ab}	83.17 ± 3.55^{f}
	3M2F	5.03 ± 0.51^{b}	92.00 ± 5.57^{g}
	3M3F	4.60 ± 0.45^{ab}	83.50 ± 4.77^{f}

*For Trial I and Trial II, different superscript letters within the columns for each parameter indicate statistically significant differences ($p<0.05$). M: MOS, F: FOS, TAB: Total aerobic bacteria, THC: Total hemocytes count. Data are presented as mean ± standard deviation.

Discussion

The findings of this study related to water quality parameters are consistent with previous studies, indicating that the environmental conditions provided during our trials were suitable for red swamp crayfish culture. Huner and Barr (1991) determined that the optimal water temperature for red swamp crayfish is 22ºC, with a pH range of 5.8-10. They observed active feeding and molting behavior at temperatures above 12ºC, but noted growth retardation when temperatures exceeded 32ºC and dissolved oxygen levels dropped below 3 mg L^{-1}. Jin et al. (2019) suggested that the optimal temperature

range for crayfish reproduction is 21-25ºC, with 25ºC being ideal for embryonic development. However, temperatures between 29-33ºC were found to cause abnormalities and mortality in embryos. Feng et al. (2021) recorded the ideal ranges for dissolved oxygen, water temperature, and pH in open systems as 3.02-7.96 mg L^{-1}, 27.2-29.1ºC, and 6.8-7.72, respectively. Yu et al. (2018) emphasized that nitrite (0-0.052 mg L^{-1}), pH (7.47-8.67), and dissolved oxygen (1.48-6.28 mg L^{-1}) levels should be maintained within these ranges for the rice-crayfish integrated culture system. Alcorlo and Baltanás (2013) reported that *P. clarkii* populations in tributaries of the Guadalquivir River (southern Spain, near the northern boundary of Doñana National Park) experienced temperature ranges of 7.3-26.5ºC (mean 19.1ºC), 1.2-28.9ºC (mean 20.05ºC), and 11-16.9ºC (mean 15.08ºC) in three different areas. In the current study, water temperatures were maintained between 20-21ºC under Mediterranean conditions (including the European and Turkish Mediterranean basins) in greenhouse or covered pond systems to minimize heating costs. Throughout both trials, the measured water quality parameters remained within the ranges reported in the literature, supporting the suitability of our small-scale culture conditions.

In this study, it was statistically demonstrated that the tested prebiotic feed additives had a positive effect on crayfish culture parameters. The positive outcomes of dietary MOS and FOS supplementation (Trial I) for red swamp crayfish nutrition are consistent with the literature, particularly when considering the dosage levels used for decapods (Genc et al. 2007; Sang and Fotedar 2010; Zhou et al. 2010; Mazlum et al. 2011; Dong and Wang 2013; Genc and Ebeoğlu 2013; Aktaş et al. 2014; Sang et al. 2014; Oktaviana et al. 2014; Selim and Reda 2015; Huynh et al. 2018; Li et al. 2018; Liu et al. 2020; Felix et al. 2020; Wee et al. 2022). Moreover, the results from the combination of additives applied in Trial II were found to be promising for future applications of prebiotic feed additives, similar to those reported by Safari et al. (2014) for *Astacus* species. One of the studies closely related to our experimental setup was conducted in 2014. In this study, Safari et al. (2014) administered *Astacus leptodactylus* crayfish with MOS and FOS prebiotics at different doses, both individually (1.5, 3, and 4.5 g kg^{-1}) and in combination (0.75, 1.5, and 2.25 g kg^{-1}) over a 126-day feeding period. Their findings indicated that the group receiving the combined prebiotic feed additives at a ratio of 2.25 MOS + 1.5 FOS showed the highest growth parameters. Li et al. (2018) applied MOS and inulin prebiotics to *Litopenaeus vannamei* species separately and in combination as feed additives for 28 days. At the end of their research, they noted that the group treated with MOS (5 g kg^{-1}) + inulin (5 g kg^{-1}) combined prebiotic additive showed higher values in terms of growth parameters compared to the other groups in which the prebiotic additives were tested separately. Li et al. (2021), on the other hand, investigated the effects of arabinoxylan oligosaccharide (AXOS) and inulin prebiotics in *L. vannamei* species at different doses (2, 4 and 8 g kg^{-1}) combined feed additive diets on shrimps for 8 weeks. As a result of their experiments, they reported that they found a significant increase in the growth parameters of the shrimps in the group fed with the feed additive applied 4 g kg^{-1}prebiotic combination.

It has been noted that studies in which different prebiotic additives are applied individually or together as feed additives in different fish species subject to aquaculture have increased in recent years (Rohani et al. 2022; Wee et al. 2022; Ye et al. 2011; Talpur et al. 2014; El-Nobi et al. 2021). When previous studies are examined, it can be stated that the co-administration of prebiotic feed additives in aquaculture leads to the promotion of beneficial bacteria in the digestive tract, which provides an advantage for aquaculture efficiency/farming performance. According to our evaluation from another point of view, the types and doses of prebiotic feed additives vary according to the species. For this reason, it was concluded that applying prebiotic feed additives at species-specific doses in aquaculture would be advantageous.

As a result of the trials carried out within the scope of our current study, in terms of growth parameters, it was determined that the application of 3 g kg^{-1} MOS with FOS both alone and in combinations increased the yield. However, according to the literature, a similar situation also shows that the ratios of FOS prebiotic feed additives vary depending on the species and application dose. When the results of whole-body nutrient component analysis obtained for Trial I and Trial II in the current study were compared in terms of protein values, the highest value for Trial I was found to be 3M 40.07% ± 0.02% and statistically different. Trial II results showed that the protein amount of 41.23% ± 0.74% in the 3M2F group was similar, whereas the control group showed the lowest value with 38.33 ± 1.02% ($p < 0.05$). These results were compatible with previous studies on prebiotic feed additives (Salem et al. 2016; Xu et al. 2022; Ali et al. 2017). It was evaluated that the results of the two trials were similar to previous studies regarding raw oil, crude ash and dry matter levels (Mazlum et al. 2011; Akbary and Jahanbakhshi 2018). The results of the nutrient component analysis revealed that prebiotic combinations increased the amount of whole-body protein.

In crayfish, the hepatopancreas is typical with its tubular structure. In the tubular system in the hepatopancreas structure, the production of digestive enzymes and the functions of digestion, absorption, and storage of nutrients such as glycogen and fat are also performed (Loizzi 1971). Prebiotics as feed additives in the digestive tract induce probiotic microorganisms. It is reported that the use of different prebiotic compounds contributes to the suppression of pathogenic microorganisms that cause diseases, in other words, the increase in the number of beneficial microorganisms, as well as the decrease in their ability to cause disease, thus improving the health conditions of the host organism (Li et al. 2007; Bosscher et al. 2009; Safari et al. 2014). In the study in which the addition of prebiotic feed additives to crayfish diets was tested alone and together, our hepatopancreas histology findings in all experimental groups showed that prebiotic feed additives did not cause any structural negativity or difference. The findings of our study are compatible with the histomorphological findings of previous studies on prebiotic feed additives (Chen et al. 2017; Genc et al. 2007; Lu et al. 2019). Arthropods generally have an innate immune system developed against potential pathogens. Hemocytes play an essential role in immune reactions due to their ability to perform phagocytosis, encapsulation, nodule formation, and cytotoxicity. In this context, they are a defense mechanism against infectious agents such as bacteria and viruses from pathogenic organisms. One of the most essential innate cellular immune functions is phagocytosis by hemocytes. The increase in the total hemocyte count is considered an immune-related marker (Liu et al. 2020). Higher total hemocyte counts were achieved with prebiotic combinations in our current trials, demonstrating that sustained induced immune elements are a usable tool for the health management of crayfish during the aquaculture period. These results are significantly similar to previous studies pointing to the increase in THC numbers obtained with prebiotic and other feed additive applications (Nedaei et al. 2019; Song et al. 2014; Safari et al. 2014).

A wide variety of microflora benefit each other in the intestinal structure of arthropods. These bacteria can contribute positively to the nutrition and health of crayfish with their digestive and secretory activities (Holdich 2002). Prebiotics, an important food source, especially for the development of bifidobacteria, affect the increase in the number of bacteria; they can also be directly beneficial to digestive enzyme activities (Hoseinifar et al. 2017). In this study, when the total number of bacteria in the digestive tract was evaluated with other measured findings, the increase in the number of bacteria was considered significant. Our findings on the total number of bacteria in the digestive tract were found to be compatible with the results obtained from the cultivation of similar organisms (Hoseinifar et al. 2011; Zhang et al. 2010; Akrami et al. 2013; Nedaei et al. 2019).

The most basic expectation in aquaculture activities is to obtain the highest quality yield from a unit area per unit of time. To meet this expectation, it has started to focus on feed additives that increase the growth and survival rate. These additives, including prebiotics, have gained increasing momentum following the limitation and prevention of antibiotic use. Primarily, since it is known that acquired immunity does not occur in arthropods, it is not possible for these creatures to be vaccinated or to develop active and long-term immunity against pathogens. At this point, ensuring the current immunity is induced for a successful breeding yield is essential. While strengthening the existing immunity, using a feed additive that does not leave residues and does not contain drugs and chemicals and the widespread use of responsible farming practices are also of great importance to obtain safe food that is not harmful to animal and human consumption. After the trials and tests, it was seen that the combined application of 3MOS (3 g kg^{-1}) and 3FOS (3 g kg^{-1}) prebiotic feed additives enabled us to achieve the targeted outputs.

In this study, the effects of MOS and FOS, which are known for their safe use in stimulating immunity and enhancing resistance, were tested on the aquaculture yield of red swamp crayfish (*P. clarkii*) for the first time (in indoor condition). The data indicated that the combined administration of these prebiotic feed additives positively influenced whole-body nutrient composition, growth parameters, hemocyte count, and bacterial count in the digestive tract. As *P. clarkii* is the second most cultivated decapod species globally, the findings from this study provide valuable insights that could contribute to the application of dietary prebiotics in red swamp crayfish culture, both for aquarium and food production purposes.

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