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Single or combined effects of fructo- and mannan oligosaccharide supplements on the growth performance, nutrient digestibility, immune responses and stress resistance of juvenile narrow clawed crayfish, *Astacus leptodactylus leptodactylus* Eschscholtz, 1823

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ABSTRACT

A 126-day experiment was carried out under controlled conditions to compare the effects of mannanoligosaccharide (MOS) and fructooligosaccharide (FOS) at three levels (1.5, 3.0 and 4.5 g kg^{-1}) in the single diets and three levels (0.75, 1.5 and 2.25 g kg^{-1}) in the combined diets on the growth performance, nutritional efficiency indices, In vivo ADC of nutrients, digestive enzymes, hemolymph indices and finally, biological responses against 12-h air exposure and 48-h *Aeromonas hydrophila* exposure challenges of juvenile (10.56 ± 0.32 g) crayfish *Astacus leptodactylus leptodactylus*. The highest values of SGR (1.59% day^{-1}), VFI (3.80% body weight day^{-1}), survival rate (94.1%) and the lowest FCR (2.67) were observed in the juvenile crayfish fed the diet containing 2.25 g kg^{-1} MOS and 1.5 g kg^{-1} FOS. The significantly ($p < 0.05$) highest PER (3.27), LER (3.45), EER (3.78), PPV (71.35%), LPV (73.25%) and EPV (89.20%) were related to the diet containing 2.25 g kg^{-1} MOS and 1.5 g kg^{-1} FOS. The mean of In vivo ADC_{OM} (73.47%), In vivo ADC_{CP} (84.98%), In vivo ADC_{CF} (83.79%) and In vivo ADC_{GE} (82.77%) of the juvenile crayfish fed the diet containing MOS was higher than those of juvenile crayfish fed the diet containing FOS. The juvenile crayfish fed the diet containing 2.25 g kg^{-1} MOS and 1.5 g kg^{-1} FOS had the significantly ($p < 0.05$) highest activities (U mg^{-1}) for amylase (8.21), lipase (7.3) and alkaline protease (7.5) and the mean ($\times 10^5$ cell ml^{-1}) of hemolymph indices ($\times 10^5$ cell ml^{-1}) including THC (107.14), HC (97.18), SGC (38.12) and LGC (47.36). After 12-h air exposure challenge, the juvenile crayfish fed the combined diets showed significantly ($p < 0.05$) higher activities of PO, SOD, LYZ and NOS than those of fed the single diets. The mean survival rate of *A. hydrophila* injected crayfish fed the single diets containing MOS (20.1%) was significantly ($p < 0.05$) higher than those of fed the single diets containing FOS (15.9%) and control (11.6%). At the levels tested, 2.25 g kg^{-1} MOS and 1.5 g kg^{-1} FOS in the diet was considered optimum. It can be concluded that dietary MOS and FOS exerted positive effects on the growth performance, feed utilization and accelerated crayfish immune responses against air and bacterial exposure challenges.

1. Introduction

Crayfish, *Astacus leptodactylus*, is the most fecund (200–400 eggs by a female) species of all native European species (Karimpour et al., 2011). The commercial value of crayfish (106–211.5 metric tonnes) was 1.5–2.5 million US $ during 2003–2009 in Iran (Karimpour et al., 2011). In Iran, *A. leptodactylus* is present with subspecies: *Astacus leptodactylus eichwaldi* in Anzali lagoon with brackish water and *Astacus leptodactylus leptodactylus* in freshwater sources. Successful introduction of *A. leptodactylus leptodactylus* into Iranian new freshwaters led to stable populations during the last decade (Karimpour et al., 2011). Crayfish has basically an omnivore habit (Franke et al., 2013). In captive conditions, there is lack of information about foraging suitable feedstuffs (animal, plant and singe cell proteins, fat sources, carbohydrates and

Abbreviations: MOS, mannanoligosaccharide; FOS, fructooligosaccharide; DP, degree of polymerization; ADC, Apparent digestibility coefficient; ADC_{CP}, ADC of crude protein; ADC_{CF}, ADC of crude fat; ADC_{GE}, ADC of gross energy; SGR, specific growth rate; VFI, voluntary feed intake; FCR, feed conversion ratio; PER, protein efficiency ratio; PPV, protein productive value; LER, lipid efficiency ratio; LPV, lipid productive value; EER, energy efficiency ratio; EPV, energy productive value; HC, the number of hyaline; SGC, the number of semi-granular; LGC, the number of large-granular; PO, phenoloxidase activity; SOD, superoxide dismutase; LYZ, lysozyme; NOS, nitric oxide synthase.

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supplements) and inclusion levels in the diet (Garza de Yta et al., 2012; Glencross et al., 2007; González et al., 2012; Safari et al., 2013). Using dietary supplements including nucleotides, probiotics, prebiotics and symbiotics is one of important options to obtain sustainable production in the aquaculture industry due to enhance non-specific defense mechanisms.

Considering key parameters such as prebiotic origin (fungi or plant), chemical structure, degree of polymerization (DP), initial weight, feeding period, supplement dose, basal diet formulation, intestinal flora, type of tested biological responses including growth performance, survival rate, carcass quality, enzymatic and immunological indices and how to set up a challenge test (e.g. physical, chemical or biological stressors) are critical to obtain a holistic approach in order to select a beneficial prebiotic in the sustainable aquaculture industry (Glencross et al., 2007; Safari et al., 2013, 2014a; Ye et al., 2011). Using MOS in the formulated diets of 1.25 g *A. leptodactylus* for 60 days (Mazlum et al., 2011), 4–94 g *Cherax tenuimannus* for 30–112 days (Sang and Fotedar, 2010; Sang et al., 2009) and 35.14 g *Cherax destructor* for 56 days (Sang et al., 2011) or FOS in the formulated diets of 15–17 g *Procambarus clarkii* for 30 days (Dong and Wang, 2013) were studied. Mazlum et al. (2011) reported that the rate of 3.0 g kg^{-1} MOS in the diet of juvenile *A. leptodactylus* showed the highest SGR (1.37% day^{-1}) and the lowest FCR (1.93) compared to other inclusion levels of MOS (0, 1.5 and 4.5 g kg^{-1}). FOS as an immunostimulant in the diet of red swamp crayfish, *P. clarkii*, increased the activities of phenoloxidase and superoxide dismutase with an increase in the dose from 2.0 to 10.0 g kg^{-1} (Dong and Wang, 2013). Short chain FOS fed with Pacific white shrimp, *Litopenaeus vannamei*, postlarvae during 42 days influenced the gastrointestinal microbiota composition and improved immune responses and disease resistance compared to control (Li et al., 2007). There is little information about combined use from MOS and FOS in the aquafeeds (Ye et al., 2011). The Japanese flounder, *Paralichthys olivaceus*, fed with a combined diet containing 2.5 g kg^{-1} FOS, 2.5 g kg^{-1} MOS and 10^{7} cell g^{-1} *Bacillus clausii* had elevated protease and amylase activities and final body weight than those of control diet (Ye et al., 2011).

There are various stressors (physical, chemical and biological) to evaluate the efficiency of feed additives (Safari et al., 2013; Stoner, 2012; Zhang et al., 2011). Despite stimulating the host immune system (Dimitroglou et al., 2011; Trichet, 2010), the most important challenge facing the use of additives such as prebiotics is the differences between values of immunological responses of treated animals before and after exposing to a stressor (Safari et al., 2013). It is questionable how to explain the results of experiments with three different values (positive, negative or without differences) obtained from subtraction of values before and after challenges. In this regard, the survival rate of treated animals is an integrated index. It is clear that up-regulation of immune responses after feeding trials with prebiotics as a new homeostatic (energetic) level is an energy-consuming process. Therefore, it must be confirmed how much difference between tendencies of immune responses can be considered as a suitable index. Although, standard conditions has not been defined for challenge tests in the feeding trials. The critical factors to set up a challenge test are type, quantity and exposure time of stressor, genetic and feeding history of target animal, molt cycle, sampling method (plasma or serum) and physicochemical conditions of rearing facilities (Hai et al., 2009; Safari et al., 2013). However, whether or not single or combination of effects of MOS and FOS can protect juvenile crayfish from different stressors remains unclear and biological responses induced by the MOS and FOS via single or combined uses in the aquatic invertebrates need additional investigation. The aim of the present study is to evaluate dose–response of MOS and FOS via single or combined inclusion levels on the growth performance, nutrient digestibility, immune responses and stress resistance of juvenile narrow clawed crayfish (*A. leptodactylus leptodactylus*).

2. Material and methods

2.1. Experimental diets

A basal diet (384.1 g kg^{-1}, crude protein; 128.5 g kg^{-1}, crude fat; 14.93 MJ kg^{-1}, Gross energy) as control diet (Ackefors et al., 1992; Safari et al., 2014a) was formulated that met all known nutritional requirements for crayfish (Table 1) with WUFFDA (Windows-Based User-Friendly Feed Formulation, done again; University of Georgia, Georgia, USA) software. Two prebiotics, mannanoligosaccharide (MOS, immunogen®, International Commerce Corporation Co., USA) and fructooligosaccharide (FOS, Raftilose® P95, Orafti Co., Belgium) were used at three doses 1.5, 3.0 and 4.5 g kg^{-1} (in the single forms) and 0.75, 1.5 and 2.25 g kg^{-1} (in the combined forms) in the place of carboxymethyl cellulose. The minimum levels of mannan in MOS and fructose in FOS guaranteed by manufacturer are 90% and 91%, respectively. DPs of mannan and fructose in MOS and FOS range from 2 to 85% and 2 to 92%, respectively. The other components are mainly glucose, fructose and sucrose. All feedstuffs were ground to a particle size <250 μm (Glencross et al., 2007). After adding fish oil, supplements and water (350 g kg^{-1}) contents, respectively, the mash was transferred from the hand pelletizer (Abzarsazan CO, Iran) with a 2 mm die, dried at 30 °C, packed in three-layer water-proof nylon bags and maintained at −20 °C (Hardy and Barrows, 2002) until feeding trials were started. Finally, sixteen experimental diets were formulated including control, 1.5 MOS, 3.0 MOS, 4.5 MOS, 1.5 FOS, 3.0 FOS, 4.5 FOS, 0.75 MOS + 0.75 FOS, 0.75 MOS + 1.5 FOS, 0.75 MOS + 2.25 FOS, 1.5 MOS + 0.75 FOS, 1.5 MOS + 1.5 FOS, 1.5 MOS + 2.25 FOS, 2.25 MOS + 0.75 FOS, 2.25 MOS + 1.5 FOS and 2.25 MOS + 2.25 FOS.

Table 1
Composition (g kg^{-1} dry matter) of the control diet fed juvenile crayfish (10.56 ± 0.32 g).

	g kg^{-1} (dry-weight basis)
Ingredient	
Menhaden fish meal[a]	90
Soybean meal[a]	278
Corn gluten[a]	99
Wheat flour[a]	267
Corn starch[b]	38
Fish oil[a]	42
Canola oil[a]	41
Soy lecithin[a]	50
Cholesterol[d]	5
Glucosamine[c]	10
Choline chloride[4] (70%)[d]	15
Vitamin C (stay)[d]	10
Vitamin premix[d,e]	20
Mineral premix[d,e]	15
Carboxymethyl cellulose[c]	19.9
Ytterbium oxide[c]	0.1
Chemical composition	
Dry matter	874.2
Crude protein	384.1
Crude fat	128.5
Crude fiber	28.9
Nitrogen free extract	420.6
Ash	37.9
Gross energy (MJ kg^{-1})	14.93
Crude fat/crude protein	0.33

[a] Behparvar Aquafeed Co, Iran.
[b] Scharloo Chemical Co, Spain.
[c] Sigma, Germany.
[d] Kimia Roshd Co. Iran.
[e] Mineral premix contains (mg kg^{-1}) Mg, 100; Zn, 60; Fe, 40; Cu, 5; Co, 0.1; I, 0.1; and antioxidant (BHT), 100. Vitamin premix contains (mg kg^{-1}) E, 30; K, 3; thiamine, 2; riboflavin, 7; pyridoxine, 3; pantothenic acid, 18; niacin, 40; folacin, 1.5; choline, 600; biotin, 0.7 and cyanocobalamin, 0.02.