Fish and Shellfish Immunology 78 (2018) 121–126

Contents lists available at ScienceDirect

Fish and Shellfish Immunology

journal homepage: www.elsevier.com/locate/fsi

Full length article

Effect of high dietary starch levels on growth, hepatic glucose metabolism, oxidative status and immune response of juvenile largemouth bass, *Micropterus salmoides*

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ARTICLE INFO

Keywords:
Starch level
Growth
Immune responses
Antioxidant capacity
M.salmoides

ABSTRACT

An experimental trial was conducted to investigate the effects of high dietary starch levels on growth, hepatic glucose metabolism enzyme, antioxidant capacity and immune responses of largemouth bass, *Micropterus salmoides*. Fish (initial body weight: 16.9 ± 0.24 g) were fed three isonitrogenous and isoenergetic semi-purified diets containing 5%, 10% and 20% wheat starch, respectively. The results indicated that fish fed 5% and 10% starch diets showed significantly better weight gain, specific growth rate (SGR), protein efficiency ratio (PER) and feed conversion ratio (FCR) compared with that fed 20% starch diet. Meanwhile, fish fed 20% starch diet had a significantly higher hepatic glycogen and muscle glycogen contents than those fed the other diets. The alanine amiotransferase (ALT) and aspartate transaminase (AST) activities, glucose and insulin contents in plasma increased significantly with dietary starch levels, whereas triglyceride content showed the opposite trend. In addition, the highest glucokinase (GK), pyruvate kinase (PK) and phosphofructokinase (PFK) activities in liver were also observed in fish fed 20% starch diet. However, both fructose-1,6-bisphosphatase (FBPase) and pyruvate carboxylase (PC) activities in liver decreased significantly as dietary starch levels increased. Moreover, the lower superoxide dismutase (SOD) and catalase (CAT), the higher malondialdehyde (MDA) contents in liver were observed in fish fed 20% starch diets. Compared to the 5% and 10% starch, the 20% starch could enhance the content of plasma nitric oxide (NO) and the activities of inducible nitric oxide synthase (iNOS) and alkaline phosphatase (ALP). Results demonstrate that the starch levels may affect growth performance and metabolic changes, which suggest that high-starch diets were inefficiently used as an energy source by *M. salmoides* juveniles. Excessive dietary starch contents could result in oxidative stress, suppress innate immunity, and thus affect the health status of *M. salmoides*.

1. Introduction

Starch is the cheapest energy source in practical diet ingredients, and is also good binder for aquatic feeds. Generally, dietary starch is mostly digested and absorbed as glucose in the gastrointestinal tract (GIT) and used to provide energy [1]. Most studies showed that the optimal dietary starch level could improve growth and feed efficiency [2–4]. However, fish appears to have a limited capacity to utilize dietary starch for energy purposes, and the ability to utilize starch varies among fish species [5]. Excess dietary starch can negatively impact fish health through metabolic disturbances and clinical signs such as hyperglycemia [5], glycogen deposition [6], liver hypertrophy [7] and histopathological [8]. Thus, high dietary starch consumption reduce the growth rate and are often accompanied by poor feed utilization, even may increase the incidence of diseases [9].

It is well-known that carnivorous fish have lower ability to utilize the dietary carbohydrate than omnivorous and herbivorous fish [1]. Unfortunately, like the case of *Seriola lalandi* [10], *Micropterus salmoides* exhibit a poor utilization of dietary carbohydrates [11]. Previous studies reported that the optimum dietary starch level of *Micropterus salmoides* ranged from 10% to 19% [12–14]. These results indicated that the inclusion of high amounts of dietary starch remains controversial, but high starch contents would result in poor growth and feed utilization of *Micropterus salmoides*. Generally, the maximum dietary inclusion level is decided based on the protein sparing effect of starch without any adverse effect on growth and physiology of the fish [1]. In addition, recent studies have demonstrated that higher dietary starch could suppress innate immune responses [15,16], and reduce the antioxidant

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https://doi.org/10.1016/j.fsi.2018.04.046
Received 4 December 2017; Received in revised form 10 April 2018; Accepted 19 April 2018
Available online 21 April 2018
1050-4648/

Table 1
Formulation and proximate chemical composition of trial diets.

Ingredient (%)	Dietary starch levels (%)		
	5	10	20
Fish meal (670 g kg^{-1} protein)	35	35	35
Casein (900 g kg^{-1} protein)	19.5	19.5	19.5
Isolated soy protein (no sugar)	5	5	5
Wheat starch	5	10	20
Fish oil	2.7	2	0.7
Soybean oil	6.8	5	1.8
Soybean lecithin	1.5	1.5	1.5
Monocalcium phosphate	1	1	1
Sodium alginate	1	1	1
Mineral premix	2.0	2.0	2.0
Vitamin premix	1.5	1.5	1.5
Choline chloride	0.2	0.2	0.2
α-cellulose	18.8	16.3	10.8
Chemical composition (%)			
Crude protein	45.1	45.1	45.0
Crude lipid	12.8	10.3	5.8
Ash	8.3	8.3	8.4
GE (MJ/kg)	16.2	16.2	16.3

capacities of fish [12,17]. To date, limited reports are available regarding the effects of dietary starch levels on plasma biochemical indices, hepatic glucose metabolism enzyme, antioxidant capacities and immune response in juvenile largemouth bass (*Micropterus salmoides*). Therefore, the objective of the present study was to investigate the response of juvenile largemouth bass to high levels of dietary wheat starch (20%), gain a better understanding of the health implications of high dietary starch in largemouth bass, and further discuss the mechanism of growth inhibition.

2. Materials and methods

2.1. Experimental diets

Three isonitrogenous (45% crude protein) and isoenergetic (16.2 MJ/kg) semi-purified diets were formulated to contain 5%, 10% and 20% wheat starch, respectively (Table 1). Fish meal, casein and isolated soy protein (no sugar) were used as protein sources, and fish oil and soybean oil was used as the lipid source. Isoenergetic diets were made by adjusting the lipid and cellulose content. All ingredients were ground through a 320 μm mesh before final mixing through a commercial food mixer and then blended with the oils. Pellets were prepared using a dry power press MUZL180 (MuYang Group, Jiangsu, China), then air-dried and stored at 4 °C until used.

2.2. Experimental procedures

Largemouth bass were obtained from a commercial farm (Zhongxian, Chongqing, China). Prior to the trial, fish were acclimated and fed a commercial feed (Guangzhou Jieda Feed Co. LTD, China) for ten days. At the start of the experiment, the fish were fasted for 24 h and weighed after being anesthetized with 0.1 g kg^{-1} MS-222 (Sigma, USA). Largemouth bass (mean initial weight:16.9 ± 0.24 g) were randomly allocated into 12 cylindrical plastic tanks (capacity: 280 L) with screened covers for the growth trial (30 fish per tank). Each dietary treatment was randomly assigned to four tanks. Fish were hand fed with a rate equalling 3–5% of wet body weight per day, three times (08:30, 12:30 and 18:00) daily for 84 days. The water was allowed to flow into each tank at 650 mL min^{-1}. During the growth period, the water temperature ranged from 25.2 to 28.5 °C, ammonia-N was < 0.46 mg L^{-1}, dissolved oxygen was above 6 mg L^{-1}, and pH was around 7.1. The photoperiod was 12 L:12 D, with the light period from 08:00 to 20:00 h.

2.3. Sample collection

At the end of the trial, fish were fasted for 24 h before harvest. Total numbers were counted, and mean body weight of fish was measured. Two fish per tank were anesthetized with overdose of MS-222 (Sigma, USA) to assess the whole body composition.

6 h after feeding, five fish from each tank were randomly selected and anesthetized with 0.1 g kg^{-1} MS-222 (Sigma, USA), and blood sample was collected from the caudal vein using a 1-mL syringe with a 27-gauge needle. Blood was centrifuged to isolate the plasma, flash frozen in liquid nitrogen, and stored at -80 °C for further analysis. The bloodless fish were then dissected to obtain viscera and liver for calculating morphological parameters. Pooled livers and pooled dorsal muscles of another four fish per tank were also immediately frozen in liquid nitrogen and stored at -80 °C until analyzed.

2.4. Chemical analysis

All chemical composition analysis of diets and whole body were conducted by standard methods (AOAC 2005). Moisture was determined by oven drying to a constant weight at 105 °C in DHG-9240A (Keelrein Instrument Co. Ltd. China). Protein was determined by measuring nitrogen (N × 6.25) using the Kjeldahl method in FOSS Kjeltec 2300 (Foss Analytical Instruments Co. Ltd. Sweden); lipid by ether extraction (without acid hydrolysis) using Soxtec; ash by combustionat 550 °C for 12 h in a muffle furnace (Shenyang Energy-Saving Electric Furnace Factory, China). Gross energy was analyzed using a Parr 1281 Automatic Bomb Calorimeter (Parr, Moline, IL, USA). Muscle and liver glycogen content were assayed using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Plasma total protein (TP), globulin, total cholesterol (TC), triglyceride (TG), glucose, calcium and phosphorus contents, alanine amiotransferase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) activities were assayed using automatic biochemical analyzer HITACHI 7100 (ISE) and attached kit (Sichuan Maker Biotechnology Co. Ltd. Chengdu, China). Plasma inducible nitric oxide synthase (iNOS) activities and nitric oxide (NO) concent were assayed using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Plasma insulin was measured by radioimmunoassay (RIA) using bonito insulin as the standard and rabbit anti-bonito insulin as antiserum, according to the method described by Gutiérrez et al. [18].

The activities of hexokinase (HK), glucokinase (GK), phosphofructokinase (PFK), pyruvate kinase (PK), pyruvate carboxylase (PC), phosphoenolpyruvate carboxykinase (PEPCK), fructose-1,6-bisphosphatase (FBPase) and glucose-6-phosphatase (G6Pase) in liver were determined using a commercial kit (SinoBest Biological Technology Co. Ltd. Shanghai, China). Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities and malondialdehyde (MDA) content in liver were assayed using a commercial kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The protein concentration of the enzyme extracts was determined according to Bradford [19]. All enzyme assays were performed in triplicate.

2.5. Statistical analysis

The experimental parameters were analyzed by one-way analysis of variance followed by Tukey's test, at 5% probability. All statistical procedures were performed with the aid of the SPSS software version 17.0 for Windows. All results were presented as the mean ± standard error (SE).