Aquaculture 489 (2018) 46–55

Contents lists available at ScienceDirect

Aquaculture

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

Targeted gene expression panels and microbiota analysis provide insight into the effects of alternative production diet formulations on channel catfish nutritional physiology

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ARTICLE INFO

Keywords:
Channel catfish, alternative feeds
PCR array
Microbiota
Gene expression

ABSTRACT

The present research evaluated targeted gene expression panels and microbiota analysis to provide greater insight into the effects of alternatively-sourced dietary ingredients on production indices, gut health, changes in the gut microbiota and genes involved in the regulation of appetite, growth, metabolism, and intestinal inflammation. Four dietary formulations were based primarily on distinguishing protein sources: (D1-MFM) menhaden fishmeal (control), (D2-MBM) porcine meat and bone meal, (D3-SBM) soybean meal, and (D4-CSM/CGM) cottonseed meal/corn germ meal, respectively, and fed to channel catfish for 12 weeks. Differences in feed conversion ratio (FCR), specific growth rate, feed intake, body condition, weight gain, proximal intestine histology, intestinal microbiota composition, and quantitative gene expression were analyzed. FCR was significantly ($P < 0.05$) increased in D2–4 relative to D1-MFM; however, other production indices were unaffected by treatment. Dietary treatment also had no effect on intestinal histology ($P < 0.05$). Effects of alternative dietary formulations on the gut microbiota were minimal, although when using Chao1, a significant effect of dietary treatment was detected ($P = 0.0497$) on gut-associated microbiota richness estimates. D3-SBM caused diet-specific differences ($P < 0.05$) in the expression of neuropeptide Y, peptide YY, and D2-MBM, D3-SBM, and D4-CSM/CGM resulted in differences in α-amylase, insulin receptor-a, glucose-6-phosphate-dehydrogenase, glucocorticoide receptor 1, and glucocorticoide receptor 2, relative to D1-MFM. These changes likely relate to differences in diet-mediated regulation of appetite and glucose metabolism, and perhaps the modulation of gut passage rate. By evaluating the molecular regulation of these pathways, as well as surveying the gut-associated microbiota, effects not detectable in short-term feeding trials may be elucidated which explain subtle differences in performance, such as FCR, as observed in the present study.

1. Introduction

Successful culturing of channel catfish *Ictalurus punctatus* requires the use of nutritionally complete diets which provide energy and nutrients at or above the minimum levels required for maximal growth and feed efficiency (Li and Robinson, 2013). A growing concern in commercial channel catfish production is the source and cost of high-quality, sustainable protein feedstuffs for use in diets. Common dietary protein feedstuffs include a range of animal and plant sources. Typical animal protein sources include menhaden fishmeal (MFM), porcine meat and bone meal (MBM), poultry by-product meal, and catfish offal meal, and are generally much more expensive than soybean meal (SBM), cottonseed meal (CSM), and distillers dried grains with solubles (DDGS), the more commonly used plant protein sources (Li and Robinson, 2013). In recent years, a key traditional ingredient in fish diets, fishmeal, has risen steeply in price in response to its increased demand in the aquafeeds industry. This has resulted in the increased use of other more moderately-priced protein feedstuffs, predominantly SBM. Although SBM remains low in cost relative to FM, SBM prices have also increased with demand, therefore alternatives are being sought to increase the profit margin for catfish production. To be sustainable and profitable, producers, nutritionists, and feed manufacturers are investigating the use of more non-traditional protein sources in catfish diets, while aiming to maintain nutritional quality and fish performance (Lochmann et al., 2012; Hardy, 2010).

One challenge the industry faces when substituting higher amounts

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https://doi.org/10.1016/j.aquaculture.2018.02.004
Received 19 October 2017; Received in revised form 8 January 2018; Accepted 2 February 2018
Available online 03 February 2018
0044-8486/

of plant protein source to replace fishmeal is addressing effects of the nutritional limitations of plant ingredients. Fishmeal contains a variety of essential nutrients, in addition to many other compounds that are of physiological importance to fish (Hardy, 2010). When fishmeal levels in diets are reduced, feed producers blend plant protein concentrates, supplemental amino acids, and vitamin and mineral premixes to overcome various deficiencies and imbalances that are detrimental to the health and growth performance of the fish, but this may not address all issues caused by plant protein sources, including the potential antagonistic interactions and antinutritional factors among plant feedstuffs that lower vitamin and mineral bioavailability (Hardy, 2010).

The majority of alternative plant-derived nutrient sources are known to contain a variety of anti-nutritional factors (Francis et al., 2001), substances that directly or indirectly affect nutrient utilization, health, and fish production, many of which are removed or deactivated during processing of the feed. An excellent example of this is the enteritis-inducing effects of SBM being removed during the process of extracting carbohydrates from SBM to create soy protein concentrate and isolate (Hardy, 2010). Other anti-nutritional factors in plant proteins, such as phytic acid, gossypol, tannins, and saponins, are not inactivated or removed during processing and pelleting and can reduce nutrient bioavailabliity, affect fish performance, or reduce feed intake (Bureau et al., 1998; Francis et al., 2001; NRC, 2011). The negative effects of these anti-nutritional factors must be managed by removal, deactivation, or supplementation.

The combination of known nutritional limitations and anti-nutritional factors of alternative protein feedstuffs warrants more investigation of their effects on fish physiology, especially as they relate to appetite, growth, metabolism, and intestinal health in channel catfish. The regulation of these systems is controlled by the expression of a number of genes in a variety of physiological pathways, and by the communities of microorganisms that inhabit the intestinal tract. Assessing the expression of genes in these key pathways, as well as monitoring any changes in the gut microbiota in response to changing dietary formulations with alternative protein feedstuffs, could help us better understand the physiological implications of these sources at a molecular level within particular pathways. This understanding is essential to developing aquafeeds that are of high nutritional quality, while striving to maintain cost-effectiveness and maximize sustainability.

2. Methods

2.1. Diet preparation

Four diets (Table 1) were formulated with practical ingredients and produced at the Thad Cochran National Warmwater Aquaculture Center (Stoneville, Mississippi, USA) and shipped to Southern Illinois University Carbondale. Experimental diets were formulated to meet or exceed the requirements of fingerling channel catfish (NRC, 2011) and mimic alternative formulations of consideration in the United States catfish industry, with the interest of reducing the cost of protein ingredients through the substitution of fish and soybean meal with less expensive meat and plant meals on a digestible protein basis. All diets were formulated to contain 32% crude protein and 6% crude lipid (as fed). Diet 1 (D1-MFM) was considered the “Positive Control” and was formulated as a high quality channel catfish feed (Robinson et al., 2001), containing MFM (80 g/kg); SBM (385.5 g/kg), and CSM (100 g/kg) as the primary protein sources. The major protein substitutions in the other three diets were as follows: D2-MBM substituted MBM for FM; D3-SBM substituted SBM for FM; and D4-CSM/CGM substituted corn germ meal (CGM) and increased CSM in replacement of FM and SBM.

Dietary ingredients were ground through a 1-mm screen with a hammer mill prior to mixing (201XLA1FC, Holmes Bros Technologies, Saint Albans, West Virginia, USA). All dry ingredients were weighed out with a top-loading balance and homogenized in a V-mixer (C436647, Patterson-Kelly Co., East Stroudsburg, PA, USA) for 20 min. Twenty grams of menhaden oil per kg were then added to each diet, and the diet was further mixed in a dough mixer (Hobart 0340, Troy, OH, USA) for 10 min. After adding distilled water (30%), the diet was again mixed in the Hobart dough mixer, then processed through a meat grinder (Hobart 4822, Troy, OH, USA), spread out on drying trays, and dried in a drying oven (13-261-28A, The Grieve Corporation, Round Lake, IL, USA) at 120 °C for 25 min. Finally, it was mixed once more in a concrete mixer (59015C, Gilson Company, Lewos Center, OH, USA) for 5 min to ensure adequate mixing. All diets were bagged, labeled, and stored in a −20 °C freezer until the start of study.

Table 1
As fed dietary formulation and analyzed proximate composition of experimental diets for channel catfish.

Experimental diets				
Ingredients (g/kg)	D1-MFM	D2-MBM	D3-SBM	D4-CSM/CGM
Carboxymethyl cellulose	20	20	20	20
Corn	234.8	215.7	191.8	167.5
Corn germ meal	0	0	0	200
Cottonseed meal (41%)[a]	100	100	100	200
Dicalcium phosphate	7.3	4.3	15.3	16
Fishmeal, menhaden (61%)	80	0	0	0
Lysine-HCL	0	0.4	0	2
Menhaden oil	20	20	20	20
Porcine Meal (65%)	0	80	0	0
Soybean Meal (48%)	385.5	407.3	500.5	372
Wheat middlings	150	150	150	0
Trace mineral premix[b]	2	2	2	2
Vitamin premix[c]	0.5	0.5	0.5	0.5
Proximate composition (g $100\,g^{-1}$)				
Dry matter	94.6	95.3	94.8	95.5
Crude protein	31.5	32.2	31.9	32.2
Crude lipid	5.8	5.9	4.9	5.0
Ash	7.1	7.3	6.9	6.6
Fiber[d]	5.8	6.0	6.1	7.7
DE (Kcal/kg)[e]	2743	2734	2670	2577

[a] Values in parentheses represent percentage protein.
[b] Contribution, mg/kg of diet: iron, 140; copper, 14; manganese, 200; zinc, 400; cobalt, 0.2; iodine, 4.8.
[c] Contribution per kilogram of diet: vitamin A, 6,600,000 IU; vitamin D, 2,200,000 IU; vitamin E, 66 mg; vitamin K, 2 mg; thiamin, 5 mg; riboflavin, 13 mg; niacin, 22 mg; pantothenic acid, 35 mg; folic acid, 2.2 mg; Vitamin B6, 11 mg; Vitamin B12, 11 μg; Vitamin C, 198 mg; selenium, 0.1 mg.
[d] Theoretical fiber content (Feedstuffs, 2016).
[e] Digestible energy; theoretical content (NRC, 2011).

2.2. Experimental design/tank set-up

The following experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Southern Illinois University Carbondale (SIUC) (Protocol number: 14-052). Channel catfish were obtained as fry from the Thad Cochran National Warmwater Aquaculture Center (Stoneville, Mississippi, USA) and cultured for three months at the Center for Fisheries, Aquaculture, and Aquatic Sciences at Southern Illinois University Carbondale. Prior to the start of the study, twenty fish were randomly stocked into each of twelve 75-L tanks in a recirculating aquaculture system (RAS) sourced with municipal water. Municipal water was treated with sodium thiosulfate ($Na_2S_2O_3$), sodium bicarbonate ($NaHCO_3$), and crystal rock salt (NaCl) to dechlorinate and maintain alkalinity and salinity. Temperature and dissolved oxygen readings were recorded daily (YSI Model 550A Dissolved Oxygen Meter, Yellow Springs, Ohio, USA), and maintained at 22 °C and $\geq 7\,mg\,L^{-1}$. Total unionized ammonia (NH_3), alkalinity, nitrite, and hardness were measured weekly using a Lamotte Smart3® Colorimeter (La Motte Company, Chestertown, Maryland,