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Assessment of the feasibility of including high levels of rapeseed meal and peanut meal in diets of juvenile crucian carp (*Carassius auratus gibelio*♀ × *Cyprinus carpio*♂): Growth, immunity, intestinal morphology, and microflora

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ABSTRACT

Two experiments were conducted to evaluate the feasibility of including high levels of rapeseed meal (RSM) and peanut meal (PNM) in juvenile crucian carp (*Carassius auratus gibelio*♀ × *Cyprinus carpio*♂) diets by investigating the growth performance, immunity, intestinal morphology, and microflora of these animals. Fish meal (FM)-based diets were used as controls in both experiments. In experiment 1, RSM and PNM replaced 250 (RSM25 and PNM25) and 500 (RSM50 and PNM50) g kg^{-1} of FM in test diets. In experiment 2, RSM and PNM substituted 500 g kg^{-1} of FM in test diets (RSM50′ and PNM50′, respectively). The diets in experiment 2 were isonitrogenous and isocaloric, and supplemental crystalline amino acids were added to compensate for the reduction in essential amino acids. More than 100 g kg^{-1} of FM was maintained in the test diets in both experiments to ensure palatability, and fish were fed these diets for 8 weeks. No significant changes in feed intake were noticed among groups in both experiments. In experiment 1, the growth rate (GR) of fish fed test diets was significantly lower than in fish fed control diets; the feed conversion ratios (FCR) were higher in fish fed test diets than in fish fed control diets with the exception of fish fed the PNM25 diet. The lower GR observed in fish fed test diets was partly due to reduced dietary energy and protein levels. No significant differences were observed in the GR of fish in experiment 2; however, the FCR of fish fed the RSM50′ diet increased, whereas the protein efficiency ratio decreased in fish of both test groups, suggesting that growth retardation would have occurred over a longer period. No significant changes were observed in serum superoxide dismutase activity in both experiments. Compared with control groups, the lymphocyte proliferation index (LPI) decreased in fish fed the RSM50 diet but not in those fed the RSM50′ diet; the LPI increased in fish fed the PNM50 and PNM50′ diets. The secretion of mucus, which formed a peritrophic membrane-like structure, was enhanced when diets included high levels of RSM and PNM. Mild intestinal histological changes, but no significant inflammatory responses or intestinal microflora changes, were observed. From these results, we came to the general conclusion that the inclusion of high levels of RSM and PNM in the diet not only retarded growth but also produced health risks to the crucian carp.

1. Introduction

The success and sustainability of aquaculture depends on the minimization of the operational cost of feed, which generally comprises 50–60% of the total cost of intensive farming. Fish meal (FM), the major ingredient of fish feed, is expensive, and the competition with other livestock industries for the available static supply of FM is increasing. As a result, plant-derived materials are increasingly being considered as alternate sources of nutrients for fish feeds (Gatlin et al., 2007). Usually, the use of plant proteins has certain limitations due to a variety of factors, including a deficiency or imbalance in essential amino acids (EAAs), the presence of antinutritional factors (AFs) or toxins, and decreased palatability. However, certain aquatic animals appear to tolerate higher levels of dietary plant ingredients than terrestrial animals. Cottonseed meal (CSM) could only be included at 100 g kg^{-1} in broiler chicken diets (Elangovan et al., 2006; Mandal et al., 2004) and at 150 g kg^{-1} in dairy cow diets (Zhang et al., 2007), whereas CSM could be included at 560 g kg^{-1} in crucian carp (*Carassius auratus gibelio*♀ × *Cyprinus carpio*♂; Cai et al., 2011) diets and at 588 g kg^{-1} in rainbow trout (*Oncorhynchus mykiss*; Lee et al., 2006) diets without producing significant negative impacts on growth and filet quality. Canola

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meal could be incorporated at levels of up to 600 g kg^{-1} in red seabream (*Pagrus auratus*, Paulin) diets (Glencross et al., 2004b) and up to 460 g kg^{-1} in channel catfish (*Ictalurus punctatus*) diets (Lim et al., 1998) without producing deleterious effects on growth. These results indicate that the high inclusion of single plant ingredients in diets is feasible for certain fish species. Even if animal production is compromised when high levels of plant ingredients are used in the diet due to nutritional imbalances, the use of these ingredients may still be acceptable in certain conditions, e.g. costs can be decreased, farmers can earn more, or no enough ideal ingredients are available. Nevertheless, an increased susceptibility to disease was observed in some fish species that were fed diets with high levels of plant ingredients, even though the animals showed normal growth and feed intake (Krogdahl et al., 2010; Sitja-Bobadilla et al., 2005). Disease can lead to huge economic losses and should be avoided. Therefore, in addition to growth performance, the potential health risks resulting from the high inclusion of plant ingredients should be studied.

Aquatic animals that have low positions in the food chain, such as omnivorous, generally performed well on diets with high levels of plant ingredients (Glencross et al., 2004a). The crucian carp (*C. auratus gibelio*♀ × *C. carpio*♂) is a widely cultured omnivorous fish species. FM had been completely replaced by plant protein sources in crucian carp commercial feed. Meanwhile, the incidence of disease in crucian carp also increased. Approximately 100 g kg^{-1} FM (or FM together with other animal ingredients) has therefore been replenished into the feed for crucian carp (Ye and Cai, 2013). However, it is still unclear whether there is a link between the utilization of plant protein sources in crucian carp commercial feed and the incidence of disease in this species.

Rapeseed (*Brassica napus*) meal (RSM) and peanut (*Arachis hypogaea*) meal (PNM) are widely available plant protein sources. These plant protein sources might be able to be used in the crucian carp diet at high levels because no decreases in feed intake, digestibility of proteins, most amino acids, energy, and protein retention were observed when RSM and peanut cake were incorporated into the diet of its female parent, gibel carp (*C. auratus gibelio*), at 629 and 495 g kg^{-1}, respectively (Xie et al., 2001). Nevertheless, RSM is rich in glucosinolates, which were observed to decrease the activity of superoxide dismutase (SOD) (Sreejith and Oommen, 2006) and the concentrations of triiodothyronine and thyroxine (Burel et al., 2001) and therefore may weaken their immunity (Hodkinson et al., 2009). Peanuts are one of the host crops that are most easily contaminated with *Aspergillus flavus*, resulting in aflatoxin contamination; contamination with this toxin at levels ranging from 0.94 to 3 mg kg^{-1} of feed was observed to produce reduced growth, reduced feed intake, and liver damage in tilapia (Haller and Roberts, 1980). Aflatoxins also led to decreases in the lymphocyte proliferation index (LPI) and immunoglobulin production in rainbow trout in response to stimulation with mitogen lipopolysaccharide (Ottinger and Kaattari, 1998). However, the female parent of crucian carp was found to be less susceptible than other species to aflatoxin B_1 (Huang et al., 2011). Moreover, it was unclear if the immunity parameters such as SOD activity and LPI would be influenced by high inclusion of PNM containing low concentration of aflatoxins.

The gastrointestinal tract is an organ with multiple roles in nutrition and immunity (Montalto et al., 2009; Rombout et al., 2011); this organ is also the first site of exposure to dietary ingredients. Therefore, the histomorphology and microflora of the gastrointestinal tract have been used to assess the safety of plant protein sources (Desai et al., 2012; Penn et al., 2011; Sissener et al., 2009). Inflammation of the intestine was observed when fish meal was replaced with plant protein sources (Baeverfjord and Krogdahl, 1996; Heikkinen et al., 2006; Uran et al., 2008), and the severity of the inflammation was dependent on the amount and type of plant protein sources used (Uran et al., 2009). The intestinal microbiota also changed when plant ingredients were included in the diet (Bakke-McKellep et al., 2007; Desai et al., 2012; Heikkinen et al., 2006; Ringø et al., 2006). However, most of the above-mentioned research focused on soybean meal (SBM), with relatively little attention given to RSM and PNM.

Based on this background, the objectives of this work were to evaluate the growth performance of crucian carp, and the potential health risks associated with the high inclusion of RSM and PNM in diets. Fish health was assessed by examining the effects of test diets on immunity (SOD activity and LPI), intestinal morphology and microflora. It is anticipated that the results will provide helpful information to the crucian carp industry.

2. Materials and methods

2.1. Diet

The FM, RSM and PNM used in this study were provided by Jiangsu Haichen Biotechnology Co., Ltd., Nantong, China. The proximate compositions of the feed were determined using the methods previously established by the Association of Official Analytical Chemists (AOAC, 1995). Briefly, moisture was determined by oven-drying the feed at 105 °C to a constant weight. Crude protein was estimated using the Kjeldahl method (KDN-04 III, Shanghai XianJian Instruments Co., Ltd., Shanghai, China) after acid digestion [LNK-872 versatile combustion, Yixing Science and Instrument Research Institute (YSIRI), Wuxi, China]. Crude lipid content was determined by the ether extraction method using a Soxtec system (YSIRI). Ash content was determined by combustion (LNK-872 versatile combustion, YSIRI) at 550 °C to a constant weight. Gross energy was analyzed using an oxygen bomb calorimeter (XRY-1C, Shanghai Changji Geological Instrument Co., Ltd., Shanghai, China). Amino acids were measured with an automatic amino acid analyzer (Sykam S-433D, Germany; separation column filled with Li+ type sulfonic acid exchange resin; reactor volume: 50 μL; column temperature: 130 °C; flow rate: 0.3 mL min^{-1}; detector wavelengths: 440 nm and 570 nm) after acid hydrolysis. The glucosinolate concentration in RSM, as determined by HPLC according to the international standard method of ISO-10633-1, was 22.4 mmol kg^{-1}. The aflatoxin B_1 concentration of PNM, as determined by ELISA test kits (Brins-LivePro Biotechnology Corporation, Beijing, PR China), was 23 μg kg^{-1}. The proximate chemical compositions and essential amino acid (EAA) contents of FM, RSM and PNM are presented in Table 1.

Two experiments were conducted in the present study. In experiment 1, a diet containing 600 g kg^{-1} of FM was designated as the control diet (Table 2, FM diet). In the test diets, 250 and 500 g kg^{-1} of FM were substituted with RSM and PNM, and the relevant diets were designated as RSM25, RSM50, PNM25, and PNM50 according to the percentage of replacement in the diet (Table 2). To ensure palatability, 100 g kg^{-1} of FM was kept in the test diets. Diets were not

Table 1
Chemical composition of the fish meal (FM), rapeseed meal (RSM) and peanut meal (PNM) (g kg^{-1}, on an as-is basis).

Chemical composition	FM	RSM	PNM
Moisture	98	116	108
Crude protein	621	375	452
Lipid	53	14	22
Ash	165	62	48
Gross energy (MJ kg^{-1})	20.1	17.5	17.9
Lys	48.7	22.4	16.8
Met	16.9	6.9	4.7
Thr	26.5	16.9	15.4
Ile	27.6	14.9	16.7
Leu	49.5	26.1	31.5
Arg	36.8	22.9	53.2
Val	32.7	19.2	17.3
His	17.6	10.6	13.2
Phe	24.2	15.0	21.7