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Effects of replacing fishmeal protein by hemoglobin powder protein on growth performance, food intake, feeding-related gene expression and gut histology of hybrid grouper (*Epinephelus fuscoguttatus* × *Epinephelus lanceolatus*) juveniles

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A B S T R A C T

An 8-week growth trial was conducted to evaluate effects of replacing fishmeal protein (FMP) by hemoglobin powder protein (HPP) on growth performance, food intake, feeding-related genes expression and gut histology of hybrid grouper (*Epinephelusfuscoguttatus* × *Epinepheluslanceolatus*) juveniles. Seven isoenergetic (340 kcal per 100 g dry matter), isoproteic (53.5% of dry matter) and isolipidic (7.5% of dry matter) experimental diets were formulated to replace 0%, 15%, 30%, 45%, 60%, 75% and 90% FMP with HPP, being abbreviated as FMP, HPP15, HPP30, HPP45, HPP60, HPP75 and HPP90, respectively. Each experimental diet was fed to triplicate groups of 30 hybrid grouper juveniles (average initial body weight: 5.27 ± 0.05 g/fish) which were stocked into small floating cages (L 120 cm × W 70 cm × H 50 cm). Experimental cages were labeled and located in seven connective 6-m^3 indoor concrete tanks (L 3 m × W 2 m × H 1 m) with 3 cages occurring in each tank. Fish were fed twice daily (08:00 and 16:00) to apparent satiation. Water quality was monitored daily.

Weight gain% (WG%), feed intake (FI), feed efficiency (FE), protein efficiency ratio (PER) and protein productive value (PPV) were significantly influenced by different dietary treatments. Analysis for the quadratic broken-line model showed that the maximum replacement level of HPP to FMP at the intercept value of WG% in diets for hybrid grouper was estimated to be 23.3%. The intercept is the value of the zero replacement. Based on the quadratic regression model at the maximum response of WG%, the replacement level of HPP to FMP was estimated to be 17.3%. The relative mRNA expression levels of agouti-related protein (AgRP), neuropeptide Y (NPY) as well as orexin genes in hypothalamus of fish were significantly decreased by high HPP replacements (45%–90%) to FM protein in diets. For gut histology, high replacement levels of HPP to FMP significantly reduced the fold height, enterocyte height as well as microvilli height of fish foregut and midgut. Generally, approximately 17.3%–23.3% FM protein in diets for hybrid grouper can be replaced by HPP without negatively affecting fish growth performance and gut development.

1. Introduction

The aquaculture industry has grown rapidly over the last decade. Likewise, marine culture is expanding worldwide, increasing the demand for feed ingredients to support production (FAO, 2012). Nutritive value of fish feed depends largely on the quality of protein of the ingredients used in the formulation of feed (Glencross et al., 2007) because the protein component of aquaculture feeds is the single most important and expensive dietary component, especially for carnivorous and marine fish which tend to have higher dietary protein requirements than freshwater fish (NRC, 2011). For feeds of many species of carnivorous and marine fish, fishmeal (FM) is still one of the primary proteins because of its known nutritional and palatability characteristics or because of the deficiency of information on FM protein replacements by other sources of protein. However, there is no realistic prospect of FM being increased in the future, and indeed, there is increasing competition for these pelagic species for direct human consumption (NRC, 2011), indicating that the global supply of FM has been limited, and

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meeting the demands of a growing industry has become challenging. Market price of FM increased in recent years due to the increased FM demand in aquaculture feed industry and exhausted marine fishery resources (FAO, 2016), and feed costs already accounted for half of the total cost of aquaculture production (Bassompierre et al., 1997). So it is of quite importance and necessity to evaluate low-FM feed formulations for target farmed species.

One credible and genuinely cost-effective approach to reducing FM reliance of fish farming is to partially or totally substitute more expensive FMP sources with economic other protein sources (Cho et al., 1974). These alternative proteins contain dietary plant and animal protein. Plant protein inclusion has normally been limited due to deficiencies in essential amino acids, anti-nutrient factors (Gomes et al., 1995). Rendered animal protein ingredients are good sources of amino acids, with high protein content, total digestible dry matter (DM) and digestible protein and energy similar to FM (Bureau et al., 1999), and thus had a great potential for the replacement of FM protein in fish feed.

However, high replacements of fishmeal protein by dietary plant and rendered animal protein perhaps affect feed palatability, and thus reduce the appetite of farmed fish. Appetite regulation occurs in the central nervous system (CNS), mainly in the hypothalamus which is a crucial tissue in the control of food intake and energy homeostasis in mammals and fish. Some of the significant neuropeptides involved in this regulation had been found, containing agouti-related protein (AgRP), neuropeptide Y (NPY) and orexin/hypocretin (Arora, 2006; Murashita et al., 2009; Sucajtys-Szulc et al., 2010; López-Patiño et al., 1999). For fish, appetite is regulated by central and peripheral appetite-stimulating (orexigenic) or appetite-inhibiting (anorexigenic) factors (Broberger, 1999), and AGRP, NPY as well as orexin belonged to the central appetite-stimulating (orexigenic) factor (Volkoff et al., 2005). NPY was considered as one of the most powerful orexigenic neuropeptide which was mainly synthesized in the arcuate neurons of hypothalamus (Edwards et al., 1999; Kalra and Kalra, 2004). Agouti-related protein (AgRP), an important neuropeptide involved in the regulation of feeding, has been identified in both mammals and fish, and it is co-expressed with NPY and functions by increasing appetite and decreasing metabolism and energy expenditure (Cerdá-Reverter and Peter, 2003; Song et al., 2003; Klovins and Schiöth, 2005; Stütz et al., 2005), influencing feed intake mainly through the antagonistic action to central melanocortin receptors (Ollmann et al., 1997). Orexin/hypocretin is expressed by neurones restricted to the perifornical nucleus and dorsal and lateral areas of the hypothalamus, and orexin neurones interact closely with other appetite-regulating neuronal systems (Williams et al., 2001).

On the other hand, the influences of high levels of FM protein replacements by alternative protein sources on gut development should also be taken into account. It is reported that in gilthead sea bream, 34% and 52% carob seed germ meal inclusions in diets made the mucosal fold of distal intestine significantly shorter and thinner (Martínez-Llorens et al., 2012). The antinutritional factors (ANFs), such as protease inhibitors, lectins, tannins and saponins existed in plant protein sources could damage the gastrointestinal tract when fish fed high plant protein based diets (Baeverfjord and Krogdahl, 1996; Krogdahl et al., 2003).

Hemoglobin powder (HP) is a by-product by separating hemoglobin from hygienically collected and quarantine qualified healthy pork blood. Because of the low-temperature processing method (spray-drying), there is little biological degradation of amino acids in the high protein (92% of dry matter) contained HP (Lee and Bai, 1997a), and the lysine and leucine contents are high while it nevertheless presents a low isoleucine concentration (Hertrampf and Piedad-Pascual, 2000). HP also has an excellent pellet binding capacity (Harimex Infomation, 1992). Regarding the good characteristics above, it can be concluded that HP has a potential as an alternative protein source for FM protein. It was reported that 50% of FM in Nile tilapia, Japanese eel feed and 100% of FM in growing common carp feed could be replaced by HP protein without negatively affecting fish growth and feed efficiency (Song et al., 1995; Lee and Bai, 1997a; Lee and Bai, 1997b), but for sea bream (*Sparusaurata*), the hemoglobin inclusion in diets produces a reduction in fish growth (Martínez-Llorens et al., 2008). These very different results above necessitate the evaluation of HP protein replacements to FM protein for fish species for which no information on the utilization of HP protein was available.

Hybrid grouper between the brown-marbled grouper (*Epinephelusfuscoguttatus*) and giant grouper (*Epinepheluslanceolatus*) has been widely cultured in China due to its increasing market demand in recent years. Some basic nutrition information on this species such as optimal dietary protein and lipid levels, the suitable dietary P/E ratio, the reference dietary amino acids profile have been established in our previous studies (Jiang et al., 2015; Jiang et al., 2016; Wu et al., 2017), but information regarding the alternative protein sources to FM in diets for hybrid grouper is still quite limited. Therefore, the present study aimed to evaluate effects of replacing fishmeal protein (FMP) by hemoglobin powder protein (HPP) on growth performance, food intake, feeding-related genes expression and gut histology of hybrid grouper juveniles.

2. Materials and methods

2.1. Experimental diets

Seven isoenergetic (340 kcal per 100 g dry matter), isoproteic (53.5% crude protein) and isolipidic (7.5% crude lipid) experimental diets were formulated to replace 0%, 15%, 30%, 45%, 60%, 75% and 90% fishmeal protein (FMP) with hemoglobin powder protein (HPP) (Table 1). These experimental diets were abbreviated as FMP, HPP15, HPP30, HPP45, HPP60, HPP75 and HPP90, respectively. The protein

Table 1
Formulations and analyzed composition of experimental diets (dry-matter basis).

Ingredients dietary treatments	FMP	HPP15	HPP30	HPP45	HPP60	HPP75	HPP90
Peruvian fishmeal (anchovy)[a]	80.45	68.38	56.32	44.25	32.18	20.11	8.05
Hemoglobin Powder[b]	0.00	8.68	17.35	26.03	34.70	43.38	52.05
Chile fish oil (Salmon)[c]	0.08	1.15	2.22	3.28	4.35	5.42	6.49
Corn starch	14.63	14.63	14.63	14.63	14.63	14.63	14.63
Vitamin mixture[d]	1	1	1	1	1	1	1
Mineral mixture[e]	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Cellulose	0.19	2.03	3.86	5.71	7.54	9.38	11.21
Taurine	1	1	1	1	1	1	1
L-Isoleucine	0.00	0.31	0.62	0.93	1.25	1.56	1.87
L-Methionine	0.00	0.17	0.35	0.52	0.70	0.87	1.05
Carboxymethyl cellulose	2	2	2	2	2	2	2
Probiotics[f]	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Analyzed composition[g]							
Dry matter %	89.3	90.5	91.5	92.3	89.1	90.9	92.8
Crude protein %	53.1	53.5	53.0	53.3	53.6	53.6	53.5
Crude lipid %	7.4	7.3	7.4	7.1	7.0	7.2	7.2
Crude ash %	16.4	14.3	12.7	10.7	8.2	6.5	4.7

[a] Yongsheng Feed Corporation, Binzhou, China; proximate composition (% dry matter): moisture, 7.2; crude protein, 66.5; crude lipid, 9.2; crude ash, 18.3.
[b] Sonac (China) Biology Co., Ltd., Wuhan, China; proximate composition (% dry matter): moisture, 5.0; crude protein, 92.5; crude lipid, 0.5; crude ash, 0.78.
[c] Blue Ocean Marine Biological Technology Co., Ltd., Rongcheng, Shandong, China.
[d] Vitamin mixture see Lin and Shiau (2003).
[e] Mineral mixture see Lin and Shiau (2003).
[f] *Bacillus subtilis*, Zhuhai Qingyu Environmental Protection Technology Co., Ltd., Zhuhai, China.
[g] Values represent means of duplicate samples.