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Pre-soaking feed pellet significantly improved feed utilization in Asian seabass (*Lates calcarifer*)

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ABSTRACT

Effects of water pre-soaking a commercial dry feed pellet on growth, feed utilization, specific activity of digestive enzymes, fecal thermal properties, hematological parameters, muscle quality and carcass composition were investigated in Asian seabass, *Lates calcarifer*. The 2 months old fish (6.02 ± 0.04 g body weight) were subjected to four dietary treatments with three replications under a completely randomized design. The dietary treatment pellets were pre-soaked with 0, 0.25, 0.5 or 0.75 (*v*/*w*) fold amounts of water per pellets, here termed soaking ratios. After rearing for three months, there were no differences in survival (95% on average) or in growth performance (specific growth rate 1.64% body weight day^{-1} on average) of the fish across the four dietary treatments ($P > 0.05$). Superior feed utilization (feeding rate, feed conversion ratio, and protein efficiency ratio) was observed in the fish receiving the last treatment. This treatment significantly increased the specific activities of chymotrypsin and lipase, but not those of pepsin, trypsin, or amylase, relative to the baseline control. An improved feed utilization was well supported by the thermal properties of feces, assessed in relation to the available nutrients. Data on hematological parameters, muscle quality and carcass composition indicated no negative effects on the fish reared with this dietary treatment. Findings from the current study indicate an optimal pre-soaking ratio of 1:0.75 *w*/*v* of pellet to water, for enhancing the feed utilization in Asian seabass.

1. Introduction

Asian seabass (*Lates calcarifer*) is a large euryhaline species that is widely distributed in the Indo-West Pacific region. Aquaculture of this species commenced in the 1970s in Thailand and rapidly spread throughout much of Southeast Asia. The global production of farmed seabass was 71,580 tonnes annually in 2014, where Thailand, Indonesia, Malaysia and Taiwan Province of China are the major producers (FAO, 2016). Since this species is carnivorous it need a high amount of protein in the diet, making production relatively expensive. Various studies have attempted to optimize dietary replacement that would reduce the feed cost, while maintaining or improving growth performance and feed utilization (Ali et al., 2016; Glencross et al., 2016; Ngo et al., 2016). An alternative route to address the same problem is to focus on the feeding protocol. In some studies feed efficiency was improved by optimized meal frequency (Biswas et al., 2010) or by intermittent feeding that enhances compensatory growth (Azodi et al., 2016). Therefore, further developments in the feeding protocols, especially in the feeding techniques, might contribute to improved seabass rearing.

Pre-soaking the feed pellets in water, prior to use in feeding, has improved the feed utilization of juvenile green turtles, *Chelonia mydas* (Kanghae et al., 2016). In Atlantic salmon (*Salmo salar*), a pre-soaked diet containing 70% dry matter had positive effects on feed intake (Oehme et al., 2014). The availability of phosphorous was also improved by pre-soaking of the feed in water (Näsi et al., 1995; Esmaeilipour et al., 2013). Improved chemical composition and physicochemical properties of pre-soaked feedstuffs in vitro corroborate such positive effects (Thongprajukaew et al., 2013), as do growth and feed utilization trials (Hossain et al., 2001; Wina et al., 2005; Thongprajukaew et al., 2015b). These findings suggest that the feed utilization of an animal can be affected by pre-soaking feedstuff prior to pelleting, or by pre-soaking feed pellets prior to feeding. Such positive effects might be pursued further by optimizing the pre-soaking parameters, in particular the mixing ratio of water and feed pellets.

Cultured fish consume dry pellets with high energy content, in contrast to their wild counterparts that consume preys with high moisture content (Buddington et al., 1997). Pre-soaking of the dry pellets provides sufficient moisture for improved hydration before the start of digestion in the gastrointestinal tract (Papadakis et al., 2008). The physiological responses related to feed utilization are generally studied through the activities of digestive enzymes (Rungruangsak-Torrissen et

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al., 2006; Thongprajukaew et al., 2011). These enzymes produced by digestive or accessory organs along the alimentary tract help digest the available food components. Increased digestive enzyme activities have been reported to correlate well with the feed utilization of animals (Wattanakul et al., 2015). In addition, feces are also used as indicators by assessing remnants of nutrients after digestion and absorption. Their thermal transition properties, detected by differential scanning calorimetry, are linked to the amounts of available or unavailable nutrients present in feces (Kanghae et al., 2016).

The aim of this study was to optimize the pre-soaking ratio of feed pellets in water when rearing Asian seabass. The ratio was restricted to above 1:0.75 *w*/*v* (feed pellets per water), in order to prevent pellet softening that would enable break-down and dispersion in water. Survival, growth performance, feed utilization, specific activities of digestive enzymes, fecal thermal properties, hematological parameters, muscle quality and carcass composition were evaluated and used as assessment criteria. The findings from the current study provide a cost-efficient simple trick to prepare the pellet feed for rearing Asian seabass. Aside from the nutritional content in diet, also the feeding protocol (including the pre-soaking trick) is important for more efficient aquaculture production, as is here demonstrated for a specific fish species.

2. Materials and methods

2.1. Fish preparation and feeding trial

Two month old Asian seabass was obtained from Trang Coastal Fisheries Research and Development Center, Trang, Thailand. The fish were acclimatized in a cement pond (4 m × 4 m) containing 1000 L seawater for two weeks. Seabass were fed to satiation using commercial feed pellets for marine carnivorous fish (Hi-grade 9773; Charoen Pokphand PCL., Samut Sakhon, Thailand) at 08.00 and 16.00 h. Subsequently, the fish screened to have similar weights (6.02 ± 0.04 g body weight) were distributed into 12 aquaria (40 cm × 90 cm × 45 cm) containing 100 L seawater. Three aquaria per treatment with fifteen fish each were set up under a completely randomized design. These fish were fed with the feed pellets prepared by soaking them in seawater in ratios 1:0.25, 1:0.5 and 1:0.75 (*w*/*v*) for 30 min, while the baseline control treatment for comparison had no pre-soaking (ratio 1:0). The proximate chemical composition of the feed pellets is shown in Table 1. Feeding regimen was initially set at 10% of body weight per day, and then the feed amount was adjusted weekly according to the actual feeding performance. The experiment was conducted for three months with 12 h: 12 h light/dark cycle, and all the fish were reared in the conditions described above. Survival was recorded daily while measurement of body weight and length was performed every other week after anaesthetization by quinaldine. Uneaten feed was collected 1 h after feeding, dried at 60 °C until constant weight, and the determined weight was used to calculate the feeding rate (FR), feed conversion ratio (FCR) and protein efficiency ratio (PER). At the end of the feeding experiment, all the fish were starved for 24 h and then were sacrificed by chilling in ice. Three individual fish from each aquarium were collected ($n = 9$ per treatment) and the sampling included stomach, intestine, white muscle (expaxial muscle under dorsal fin) and whole carcass from the various treatment groups. Pooled fish feces and pooled blood ($n = 3$ per treatment) were also sampled. Growth performance and feed utilization were calculated as follows:

Survival (%) = [Final fish number/initial fish number] × 100

Condition factor (CF, g cm^{-3}) = [Live body weight (g)/total body length (cm)^3] × 100

Specific growth rate (SGR, % body weight day^{-1}) = [($\ln W_t - \ln W_0$)/($t - t_0$)] × 100 where W_t = mean weight (g) at day t, W_0 = mean weight (g) at day t_0.

Stomasomatic index (SSI, %) = [Wet weight of stomach (g)/wet body weight (g)] × 100

Intestosomatic index (ISI, %) = [Wet weight of intestine (g)/wet body weight (g)] × 100

Hepatosomatic index (HSI, %) = [Wet weight of liver (g)/wet body weight (g)] × 100

FR (% BW day^{-1}) = $C/[(W_0 + W_t)/2]/t \times 100$ where C = daily feed consumption (g), W_0 = initial body weight (g), W_t = final body weight (g), t = feeding duration (day).

FCR (g feed g gain^{-1}) = Dry feed consumed (g)/wet weight gain (g)

PER (g gain g protein^{-1}) = Wet weight gain (g)/protein intake (g)

2.2. Proximate chemical compositions of experimental diets

The chemical compositions of the experimental diets were analyzed for moisture, crude protein, crude lipid, crude ash and crude fiber, according to standard methods of AOAC (2005). All the analyses were performed in triplicates and are reported in % dry matter (DM). Nitrogen free extract (NFE) was calculated from 100 – (crude protein + crude lipid + crude ash + crude fiber).

2.3. Water quality management

The water quality was controlled by a recirculating aquaculture system at a flow rate of 2 L min^{-1} and supplied by air pump. The quality of water was measured weekly at the same time of day (10.00 h). The parameters monitored included temperature (Hg thermometer), pH (pH meter), total alkalinity (titration method) and ammonia (phenate method), determined according to standard methods of APHA, AWWA and WPCF (1998). Nitrite was analyzed according to the method of Strickland and Parsons (1972). Dissolved oxygen was determined by water analyzer (Multiparameter Display System; YSI 650MDS, YSI Incorporated, Ohio, USA). The water quality during the experiment is summarized as: 28.91 ± 0.30 °C, pH 7.71 ± 0.04, 6.17 ± 0.04 mg L^{-1} dissolved oxygen, 140.01 ± 3.81 mg L^{-1} alkalinity, 1.70 ± 0.25 mg L^{-1} ammonia and 0.50 ± 0.01 mg L^{-1} nitrite.

2.4. Digestive enzyme determination

2.4.1. Digestive enzyme extraction and protein quantification

Stomach and intestine (including pyloric ceca) were removed on ice, weighted and then homogenized in 10 mM Tris-HCl pH 7.5 or in 0.2 M phosphate buffer at pH 8 (1:3 *w*/*v*), respectively, using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA). The homogenate was portioned and centrifuged at 15,000 × *g*, at 4 °C for 30 min. The supernatant was collected and aliquot kept at −20 °C until use. The protein concentration of a crude enzyme extract was compared to a standard curve of bovine serum albumin (BSA), according to the standard method of Lowry et al. (1951).

2.4.2. Digestive enzyme assays

Stomach extract was only used for assaying pepsin activity (EC 3.4.23.1) while intestinal extract was for activities of trypsin (EC 3.4.21.4), chymotrypsin (EC 3.4.21.1), α-amylase (EC 3.2.1.1) and lipase

Table 1
The proximate chemical composition of experimental diet for rearing Asian seabass. The values represent triplicate determination.

Chemical composition	Soaking ratio			
	0	0.25	0.5	0.75
Moisture (% FW)	7.92	24.30	37.28	45.17
Crude protein (% DM)	46.68	46.32	45.98	46.02
Crude lipid (% DM)	8.08	8.03	7.96	8.04
Crude ash (% DM)	11.15	11.10	10.97	11.05
Crude fiber (% DM)	2.94	3.05	3.02	2.87
Nitrogen free extract (% DM)	31.15	31.50	32.07	32.02

FW, fresh weight; DM, dry matter.