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Growth performance, digestion and metabolism to fish meal replacement by rice protein concentrate in Chinese soft-shelled turtle *Pelodiscus sinensis*

C.X. Sun[b,c], D.D. Zhang[c], W.B. Liu[c], W.C. Cai[c], Y. Qian[c], K.Z. Wang[c], X.F. Li[c], G.Z. Jiang[c], W.N. Xu[a,c,*]

[a] School of Agriculture and Biology, Department of Animal Science, Shanghai Jiao Tong University, Shanghai, People's Republic of China
[b] Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, People's Republic of China
[c] Laboratory of Aquatic Nutrition and Ecology, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, People's Republic of China

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ABSTRACT

An 8-week feeding trial was conducted to evaluate growth performance, whole-body composition, metabolism and digestion of Chinese soft-shelled turtle *Pelodiscus sinensis* fed with fish meal replacement by rice protein concentrate and squid paste supplementation diets. Three isonitrogenous and isoenergetic practical diets were formulated with the inclusion of 60% fishmeal (CT), 42% fish meal +18% rice protein concentrate (RP) and 42% fish meal +18% rice protein concentrate +1% squid paste (feeding attractant) (RPS), respectively. Microcapsule lysine was supplemented in RP and RPS diets to balance the amino acid profile. Turtles (initial weight 30.65 g) were fed thrice (6:00, 12:00 and 18:00) daily to apparent satiation. The results showed that growth performance in RP group showed no significant difference with that in CT group ($P > .05$). In addition, daily squid paste supplementation significantly improved growth performance, nutrients retention and relative feed intake ($P < .05$). Significantly increased activities of hepatic alanine aminotransferase as well as intestinal protease, γ-glutamyl transpeptidase, aminopeptidase N and carboxypeptidase A were also observed in RPS group ($P < .05$). The results suggested that rice protein concentrate could replace 18% dietary fish meal in *Pelodiscus sinensis* with microcapsule lysine supplementation. Besides, diet with squid paste inclusion could improve feed intake and growth performance of *Pelodiscus sinensis*.

1. Introduction

With the decline of worldwide capture fisheries, aquaculture supplies an increasing proportion of food fish, 42.2% of the total in 2012. It has been the fastest growing animal food-producing sector globally for over half a century, with production growing by an average rate of 8.1% per year since 1961 (FAO, 2014; Tacon, 2010). However, fish meal served as a major protein sources in aquaculture has in fact reached its sustainable limits (Tacon and Metian, 2009a, 2009b). As a result, considerable attention has been devoted to the evaluation of plant proteins as the alternative sources of fish meal because of their low price and consistent nutrient (Amaya et al., 2007). Among the candidates of the plant protein sources, rice protein is considered to have great potential for the comparable protein and lipid values to fish meal (Palmegiano et al., 2006), appropriate amino acid profile and hepatoprotective effects (Han et al., 2016; Oujifard et al., 2015). Rice protein concentrate as an ingredient for aquafeed has been evaluated on fish and shrimp (Oujifard et al., 2012; Oujifard et al., 2015; Palmegiano et al., 2006; Palmegiano et al., 2007). Nevertheless, like most plant proteins, rice protein is short of relatively small soluble molecules, which are stated as palatability enhancers widely existing in marine origins (NRC, 2011). Thus, researches on feed stimulant selection were proceeded to undertake. Squid paste is processed from the organic wastes, such as hepatopancreas, mantle and digestive glands, and has been confirmed to be effective to improve feed intake and growth of fish and shrimp (Amaya et al., 2007; Hua et al., 2015; Santoso et al., 2013). Hence, to further expand the utilization of rice protein, squid paste may be appropriate to be supplemented in the diets as an effective stimulant.

Chinese soft-shelled turtle (*Pelodiscus sinensis*) has been extensively

Abbreviation: AKP, Alkaline phosphatase; APN, Aminopeptidase N; CPA, carboxypeptidase A; CT, Group fed diet included 60% fish meal; DDGS, distillers dried grains with soluble; DPP-IV, dipeptidyl peptidase IV; GOT, liver aspartate aminotransferase; GPT, alanine aminotransferase; RP, Group fed diet included 42% fish meal + 18% rice protein concentrate; RPS, Group fed diet included 42% fish meal + 18% rice protein concentrate + 1% squid paste; SDH, succinate dehydrogenase; XOD, xanthine oxidase; γ-GT, γ-glutamyl transpeptidase

* Corresponding author at: School of Agriculture and Biology, Shanghai Jiaotong University, No.800 Dongchuan Road, Shanghai 200240, People's Republic of China.
E-mail address: xuweina@sjtu.edu.cn (W.N. Xu).

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cultured in Asia for a long time because of its high nutritional value coupled with the profitable economic returns. In recent years, the demand of this species has increased remarkably due to its notable contents of both minerals and n-3 highly unsaturated fatty acids. In 2012, Chinese soft-shelled turtle production had reached up to 331,424 tons in China (China Fishery Statistical Yearbook, 2013). However, nowadays aquaculture of *P. sinensis* has been restrained greatly by high feed cost since the commercial diet of this species usually contains approximately 60% fish meal. Therefore, decreasing fish meal content in diets is highly needed.

So far, there have been little reports regarding fish meal replacement by rice protein and squid paste supplementation in the diet of *P. sinensis*. In this context, the objective of the present study was to evaluate the potential of partial replacement of fish meal by rice protein and squid paste supplementation in the diet of juvenile Chinese soft-shelled turtle.

2. Materials and methods

2.1. Animal ethics

The care and use of animals followed Animal Research Institute Committee guidelines of Nanjing Agriculture University, China. This study has been approved by the Committee of the Animal Research Institute of Nanjing Agricultural University, China (permit number: SYXK (Su) 2011-0036).

2.2. Experimental ingredients and diets

Three isonitrogenous (47% crude protein) and isoenergetic (18 MJ kg^{-1} gross energy) diets were formulated in this study. The control diet included 60% fish meal (CT), while 30% fish meal was substituted with rice protein concentrate (RP and RPS) in the test diets. RPS diet differed from RP diet by the supplementation of 1% squid paste. This account of squid paste was determined by the best diet preference of Chinese soft-shelled turtle (Sun et al., 2017). To maintain the balance of essential amino acid profile, microcapsule lysine was supplemented in the experimental diets. Formulation and proximate composition of the experimental diets were presented in Table 1, and the amino acid profiles of white fish meal, rice protein concentrate and experimental diets were showed in Table 2. White fish meal, rice protein concentrate, soybean protein concentrate, soybean meal and distillers dried grains with soluble (DDGS) served as protein sources, and crude protein content in the experimental diets was fixed to meet the requirement of this species (Nuangsaeng and Boonyaratapalin, 2001). Fish oil was supplemented as lipid sources, α-starch was used as the only carbohydrate source and squid paste was supplemented as the stimulant. In addition, minor adjustments of α-starch content were made to balance the formulations.

All the experimental diets were prepared in the laboratory. Dry ingredients were ground through a 60-mm mesh. The fine powder was carefully weighed and then mixed thoroughly with oils. After the oil was dispersed, the mash feed was packed in plastic bags and stored at −20 °C until used. Prior to feeding, 0.3 L water was added per kg of mash. The mixture was then extruded through a meat grinder and the soft pellets were offered to soft-shelled turtles.

2.3. Turtles and the feeding trial

Juvenile soft-shelled turtles were obtained from a commercial farm in Nanjing (Jiangsu, China). After two weeks of acclimation, turtles of similar size (average 30.65 g) were randomly distributed into 12 concrete tanks (2.0 m × 2.0 m × 0.8 m) at a rate of 50 turtles per tank. Three experimental diets were randomly allotted to turtles with quadruplicate tanks. Turtles were fed thrice daily (6,00, 12:00 and 18:00) for 8 weeks. The feeding amount of the soft pellets was approximately 3% of the body weight daily. This ration size was chosen based on the amount of diet consumed in 1 h by turtle during the acclimation period. At each feeding, uneaten feed was carefully collected and weighed to calculate the total feed intake. Body weights of turtles were measured every 2 weeks and the daily feed allowance was adjusted accordingly.

Table 1
Composition of experimental diet (% dry matter basis).

	CT	RP	RPS
Ingredients			
White fish meal[a]	60.0	42.0	42.0
Rice protein concentrate[b]	0.0	18.0	18.0
Soybean protein concentrate[c]	7.0	7.0	7.0
Soybean meal[d]	4.0	4.0	4.0
DDGS[e]	4.0	4.0	4.0
Fish oil[a]	1.2	1.2	1.2
α-starch[f]	21.1	19.6	18.6
CaH_2PO_4	1.4	1.4	1.4
Vitamin and mineral premix[a]	1.3	1.3	1.3
Microcapsule lysine[g]	0.0	1.5	1.5
Squid paste[h]	0.0	0.0	1.0
Proximate composition (%, dry-matter basis)			
Crude protein	46.8	46.7	47.3
Crude lipid	6.5	6.5	6.9
Crude ash	15.2	12.0	11.6
Energy (MJ kg^{-1})	18.1	18.3	18.3

Note: CT, diets including 60% fishmeal; RP, diets including 42% fishmeal; RPS, diets including 42% fishmeal and 1% squid paste.

[a] Fishmeal obtained from Tech-bank Co., Ltd. (Ningbo, China), crude protein 65%.

[b] Obtained from Hubei Jingyuan Mountain Biotechnology Co., Ltd. (Jingmen, China).

[c] Obtained from Ruilin Biotechnology Co., Ltd. (Shanghai, China).

[d] Soybean meal provided by Zhengchang Feed Industry Co., Ltd. (Jiangsu, China), crude protein 43%.

[e] Distillers dried grains with soluble, obtained from Qilong Biotechnology Feed Co., Ltd. (Shandong, China).

[f] Obtained fromYinhe Dextrin Co., Ltd. (Zhengzhou China).

[g] Containing 38% lysine was provided by Hainachuan Pharmaceutical Co., Ltd. (Foshan, China).

[h] Obtained from Yancheng Evergreen Conglomerate Co., Ltd. (Yancheng, China).

Table 2
Amino acid profiles of white fish meal, rice protein concentrate and experimental diets (% dry matter basis).

	White fish meal	Rice protein concentrate	CT	RP	RPS
Essential amino acids					
Thr	2.9	2.5	2.1	1.9	1.9
Val	3.0	3.5	2.2	2.2	2.4
Met + Cys	2.7	3.4	1.8	1.8	1.8
Ile	2.6	2.6	2.0	1.9	1.9
Leu	4.8	5.3	3.6	3.5	3.5
Phe + Tyr	4.9	6.9	3.6	3.8	3.8
Lys	5.1	1.9	3.5	3.5	3.4
His	1.3	1.5	1.0	1.0	1.0
Arg	4.3	5.0	3.1	3.1	3.1
Non-essential amino acids					
Asp	6.1	5.9	4.6	4.3	4.3
Ser	3.1	3.3	2.3	2.2	2.2
Glu	9.0	11.8	6.9	7.0	7.2
Gly	4.5	2.8	2.8	2.7	2.9
Ala	4.1	3.9	2.7	2.7	2.8
Pro	3.0	2.2	2.3	2.1	2.1
Total amino acid	61.4	62.5	44.5	43.7	44.3
Crude protein	67.1	66.5	46.8	46.7	47.3

Note: CT, diets including 60% fishmeal; RP, diets including 42% fishmeal; RPS, diets including 42% fishmeal and 1% squid paste.