Dietary squid paste supplementation promotes feed intake via brain-gut dynamic response in Chinese soft-shelled turtle *Pelodiscus sinensis*

Cunxin Sun[1,2], Yu Qian[2], Wenbin Liu[2], Weina Xu[2,3], Kaizhou Wang[2] and Bo Liu[1]

[1] Key Laboratory of Freshwater Fisheries and Germplasm Resources Utilization, Ministry of Agriculture and Rural Affairs, Freshwater Fisheries Research Center, Chinese Academy of Fishery Sciences, Wuxi, China
[2] Laboratory of Aquatic Nutrition and Ecology, College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, China
[3] School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai, China

Corresponding authors
Weina Xu, xuweina@sjtu.edu.cn
Bo Liu, liub@ffrc.cn

ABSTRACT

Background. As the primary source of protein for aquaculture, fishmeal has reached the extremity of sustainable development, our previous studies have proven that rice protein concentrate and squid paste are outstanding protein source and stimulant for *Pelodiscus sinensis*. However, little attention has been given to the molecular mechanism of the appetite modulated by the dietary nutrient factor, especially for a reptile. Thus, the present study aimed to evaluate feed intake and brain-gut dynamic responses to dietary rice protein concentrate and squid paste in Chinese soft-shelled turtle *Pelodiscus sinensis*.

Methods. Three isonitrogenous and isoenergetic practical diets were formulated including 60% fishmeal (CT), 42% fishmeal + 18% rice protein concentrate (RP) and 42% fishmeal + 18% rice protein concentrate + 1% squid paste (RPS), respectively. Microcapsule lysine was supplemented in RP and RPS diets to balance the amino acid profile. Turtles (initial weight 30.65 ± 0.97 g) were fed three times daily to apparent satiation. After the 8-week feeding trial, the turtles were exposed to 48h food deprivation, then the dynamic expression of the orexigenic and anorexigenic peptides were measured.

Results. The results showed that no significant effect was observed on feed intake when fishmeal was replaced by rice protein concentrate ($P = 0.421$), while significantly improved feed intake was found by squid paste supplemented ($P = 0.02$). The mRNA expression of anorexigenic peptides, such as leptin receptor, insulin receptor, pro-opiomelanocortin, cocaine and amphetamine-regulated transcript, cholecystokinin (and its receptor) and glucagon-like peptide-1 receptor in the brain increased significantly at 3 h past feeding ($P < 0.05$), and then decreased. Nevertheless, neuropeptide Y and peptide YY mRNA expression showed the valley at 3h and peak at 12h past feeding. Intestinal cholecystokinin receptor and glucagon-like peptide-1 receptor mRNA expression showed no difference during the postprandial time ($P > 0.05$). The results suggested that squid paste is an outstanding stimulant for *Pelodiscus sinensis*. Furthermore, the orexigenic and anorexigenic peptides evaluated here might play an essential role in short-term fasting to this species, of which the dynamic expression levels were regulated by squid paste.

INTRODUCTION

According to *FAO (2014)*, 23,000,000 excess tons of aquatic production was needed to meet current consumption by 2030. Nevertheless, as the primary source of protein for aquaculture, fishmeal has reached the limit of sustainable development (*Tacon & Metian, 2009a*; *Tacon & Metian, 2009b*). Hence, considerable efforts have been made to overcome this limitation. On account of the low price and stable nutrition, plant protein has been considered a promising protein source (*Amaya, Davis & Rouse, 2007*). Among plant protein-derived candidate species, rice protein has a comparable value of protein and lipid level to fishmeal (*Palmegiano et al., 2006*), as well as a balanced amino acid profile (*Oujifard et al., 2015*). Rice protein concentrate has been assessed as a protein source on aquatic species for years (*Oujifard et al., 2012*; *Oujifard et al., 2015*; *Palmegiano et al., 2006*; *Palmegiano et al., 2007*). However, unlike most marine sources, rice protein lacks small soluble molecular palatable stimulants (*NRC, 2011*). Hence, it is particularly necessary to improve the palatability of the feed containing rice protein. Squid paste is processed from the organic wastes in squid, and is useful in improving food intake and growth of aquatic species (*Amaya, Davis & Rouse, 2007*; *Hua et al., 2015*; *Santoso, Ishizuka & Yoshie-Stark, 2013*). Thus, to further improve the use of rice protein, squid paste can be appropriately added as an effective enhancer of appetite.

Appetite is controlled by a complex system, in which the gut-brain axis regulates central and peripheral signaling response to nutrient intake. The gastrointestinal tract releases satiety and adiposity signals, including gastric distention and satiation peptides (*Powley & Phillips, 2004*) such as cholecystokinin (CCK), peptide YY (PYY) and glucagon-like peptide-1 (GLP-1). The signals reach the solitary nucleus (SN) in the caudal brainstem through the vagus nerve. Afterward, the satiety signals combine with the obesity signals (leptin and insulin), as well as multiple hypothalamic and supra-hypothalamic input signals from the NTS afferent fiber projection to the arcuate nucleus (ARC), which forms a complex neural circuits network. Finally, the individual's reaction to a meal initiates (*Valassi, Scacchi & Cavagnini, 2008*). As for the neuropeptide system, "first-order" neurons located in ARC secrete the orexigenic neurons, such as co-expressing neuropeptide Y (NPY) and agouti-related peptide (AGRP). These neurons have since been called AGRP neurons, which are inhibited by both insulin and leptin (*Cowley et al., 2001*; *Spanswick et al., 1997*), but activated by ghrelin (*Cowley et al., 2003*). Adjacent to AGRP cells in the ARC is neurons that expressing anorexigenic pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART). Contrary to AGRP neurons, POMC neurons are stimulated by leptin and are inhibited in the absence of leptin (*Fan et al., 1997*; *Schwartz et al., 1997*). Other brain regions controlling food intake are "second-order", including the paraventricular nucleus (PVN), the lateral hypothalamus (LHA) and perifornical area

(PFA). Both gastrointestinal and neuropeptide systems acting in synergism maintain the food intake and energy balance.

Nevertheless, the regulation of food intake on the aquatic animal is quite limited, and the actual research is mainly focused on the gene cloning. Sporadic research indicated that brain-gut peptides in aquatic animals might play a different role in regulatory pathways compared to that in mammals (*Cao et al., 2011*; *Mommsen, 2000*; *Riley et al., 2008*). There is little research on functional analysis of the appetite peptides. As the most critical nutrient to the aquatic animals, protein plays a prominent role in ingestion, growth, and self-healing. Both the amino acid profile (*López et al., 2010*) and protein level (*Coutinho et al., 2012*) could affect the food consumption and the expression of appetite peptide, but the opposite results were often observed in the limited reports of aquatic animal (*Hevrøy et al., 2008*; *Sissener et al., 2013*). Hence, it is particularly necessary to explore the regulatory mechanism of aquatic animals.

Chinese soft-shelled turtle (*Pelodiscus sinensis*) has been cultivated in Asia for many years because of its high nutritional value and excellent economic benefits. In 2017, *Pelodiscus sinensis* output had been up to 344,529 tons in China (*China Fishery Statistical Yearbook, 2018*). Nevertheless, the industry is limited by the high feed cost because the cultivation of this species is dependent mainly on fishmeal. Therefore, it is necessary to reduce dietary fishmeal content in this species.

To date, there have been few reports of dietary fishmeal substitution by rice protein and squid paste supplementation in *Pelodiscus sinensis*. Furthermore, less attention has been given to the molecular mechanism of the appetite modulated by the dietary nutrient factor, especially for a reptile. In this context, this study aimed to evaluate feed intake and brain-gut dynamic responses to dietary rice protein concentrate and squid paste in Chinese soft-shelled turtle and shed more light on the molecular mechanism of the appetite when nutrients changes and particularly food is deprived.

MATERIAL AND METHODS

Animal ethics

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

Experimental feeds

Three isonitrogenous (47% crude protein) and isoenergetic (18 MJ/kg gross energy) diets were formulated in the present study. The control diet contained 60% fishmeal (CT), while 18% of fishmeal was replaced by rice protein concentrate (RP and RPS) in the test diets. RPS diet was different from the RP diet by the inclusion of 1% squid paste. This concentration of squid paste was determined by the optimal dietary preference of *Pelodiscus sinensis* (*Sun et al., 2018a*; *Sun et al., 2018b*). To maintain the consistencies of essential amino acid profiles in the experimental diets, microcapsule lysine was supplemented according to the
Table 1 Composition of experimental diet (% dry matter basis).

Ingredients	CT	RP	RPS
White fish meal	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

Notes.

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

[a]Obtained from Tech-bank Co., Ltd (Ningbo, China).
[b]Obtained from Hubei Jingyuan Mountain Biotechnology Co., Ltd (Jingmen, China).
[c]Obtained from Ruilin Biotechnology Co., Ltd (Shanghai, China).
[d]Obtained from ZhengChang Feed Industry Co., Ltd (Huaian, China).
[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).

essential amino acid profiles of the CT diet. The formulation and proximate composition of the experimental diets were presented in Table 1.

All the ingredients were ground through a 60-mm mesh. The fine powder was carefully weighed, then lipid sources, and 30% of water was added to the mixture that was further blended to ensure homogeneity. A Laboratory pelletizer was used for the pelletizing process. After drying in the laundry drier, the feeds were offered to turtles.

Turtles and the feeding trial

Juvenile soft-shelled turtles were provided by a commercial farm (Nanjing, China). The feeding trial was conducted from June to July. Turtles were cultured in concrete tanks outside and fed with CT diet for acclimation. After 2-week domestication, turtles of similar size (average 30.65 ± 0.97 g) were randomly distributed into 12 concrete tanks (2.0 m × 2.0 m × 0.8 m), 50 turtles per tank. Three experimental feeds were randomly assigned to turtles with quadruplicate tanks. Management of the feeding trial was conformed to the method of *Sun et al. (2018a)* and *Sun et al. (2018b)*. Feed pellets were put on a sedentary plate under the water. Turtles were fed approximately 3% of their body weight thrice daily (6:00, 12:00, and 18:00) for eight weeks. This ration was a little bigger than the amount of diet consumed by turtles in 1 h. At each feeding, the uneaten feed was carefully collected

by siphoning, dried, and weighed to calculate the total feed intake during the feeding trial. Bodyweight of turtles was measured every two weeks, and the daily feed allowance was adjusted accordingly. Water temperature ranged from 28 to 30 °C, pH fluctuated between 7.2 and 7.4, dissolved oxygen was maintained above 5.0 mg/L, and total ammonia nitrogen and nitrite were kept <0.2 and 0.005 mg/L, respectively, during the feeding trial.

Sample collection

At the end of the feeding trial, cumulative feed intake per turtle during the feeding trial was calculated as follows: feed intake = cumulative feed consumption/turtle amounts. To determine the effect of short-term food deprivation on gastrointestinal and neuropeptidergic mRNA expression, the turtles were exposed to 48 h food deprivation and then refed *ad libitum* for one hour. We sampled before refeeding (F) and at 3, 6, 12, and 24 h during the postprandial period. Four turtles from each treatment were randomly selected and anesthetized in diluted MS-222 (tricaine methanesulfonate; Sigma) at the concentration of 100 mg/L. The total brain (forebrain, hindbrain, and midbrain) and duodenum were sampled and stored at −80 °C for subsequent analysis. The duodenum begins with pylorus and ends at the suspensory muscle. The turtles were checked before the postprandial harvest. If there were no chyme in its stomach, the turtle would be discarded. We sampled quadruplicate each group per depot. Four operators were allotted to harvest simultaneously to avoid possible variations in mRNA dynamic expression associated with sampling time. Sampling lasted less than 10 mins per group.

RNA isolation and RT-qPCR analysis

Total brain and duodenum were used for RNA isolation. Total RNA was isolated using RNAiso Plus (Takara Co. Ltd, Japan), and then purified with RNase-Free DNase (Takara Co. Ltd, Japan) to avoid genomic DNA amplification. Purity and concentration of RNA were measured using a NanoDrop (DN-1000, Thermo Scientific, USA). After normalizing the concentration of the RNA samples, cDNA was generated from 500 ng DNase-treated RNA using ExScript^{TM} RT-PCR kit according to the manufacturer's directions (Takara Co. Ltd, Japan).

The cDNA samples were analyzed by a real-time quantitative detector (BIO-RAD, USA) using the SYBR Green II Fluorescence Kit (Takara Co. Ltd, Japan). The fluorescent qPCR reaction solution consisted of 10μL SYBR® premix Ex Taq^{TM}, 0.4μL ROX Reference Dye II, 0.4 μL PCR forward primer (10 μM), 0.4 μL PCR reverse primer (10 μM), 2.0 μL RT reaction (cDNA solution), and 6.8 μL dH$_2$O. All RT-qPCR primers were designed using the Primer 5 software and listed in Table 2. The thermal profile was 95 °C for 30s, followed by 40 cycles of 95 °C for 5s and 60 °C for 30s, followed by a melt curve analysis of 15s from 95 to 60 °C, 1min for 60 °C, and then up to 95 °C for 15s. Glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was selected as the housekeeping gene to normalize our samples because of its stable expression in fasting turtle samples in the present study. Values for the threshold (C$_T$) from the treated and control tissue templates were compared, and the $2^{-\Delta\Delta CT}$ method was used as the relative quantification calculation method (*Livak & Schmittgen, 2001*).

Table 2 Nucleotide sequences of the primers used for real-time quantitative PCR.

Gene	GenBank acc. no.	Primer sequences (5′-3′)	Tm (°C)	Amplicon length (bp)
LeptinR	XM_006125027.2	GCCTGCAGGGAATTGGCATA	62	168
		ACAGGCTCCCCACTTGATCG	64	
INSR	XM_014575357.1	ACAACCTCACCATAGCACGG	60	119
		GTCATTCCTCTCCTGACGCC	60	
NPY	XM_006138369.2	TGTCCGTGCTGCCTTTCG	61	146
		GTTGATGTAGTGCCTCAGTGC	59	
POMC	NM_001286918.1	ATGGAACTGGACTACCCCGA	60	91
		TTCCGTCTTTCTTCTCCGCC	60	
CART	XM_014574133.1	ATCGGGAAACTCTGCGACTG	60	121
		AAGGTCACTGGGTCCGTTTG	60	
GLP1R	XM_014575235.1	CAGGGACTGATGGTGGCAAT	60	212
		TAGCTGAATGTGGCTTGGCA	60	
CCK1R	XM_006138180.2	GCAGCAGCACCAAAGTAGAC	59	169
		TCTGCTGATGCGGTGTCAAA	60	
CCK	XM_006131816.2	CCTACACACAGGATAAACG	56	222
		AAACTGACACCAACACAAG	56	
PYY	XM_006118327.1	TCTCTGCCCTCCGACATTAC	59	126
		CGTACCGTGATCTGCCATTG	59	
GAPDH	NM_001286927.1	AGAACATCATTCCAGCATCCA	60	227
		CTTCATCACCTTCTTAATGTCGTC	62	

Notes.
LeptinR, leptin receptor; INSR, insulin receptor; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; CART, cocaine and amphetamine-regulated transcript; CCK, cholecystokinin; CCK1R, cholecystokinin receptor 1; GLP1R, glucagon-like peptide-1 receptor; PYY, peptide YY; GAPDH, Glyceraldehydes-3-phosphate dehydrogenase.

One-way analysis of variance (ANOVA) was used to investigate the feed intake. Levene test was used to test the homogeneity of variances. If significant differences were observed ($P < 0.05$), the means were ranked by Tukey's multiple range test. Two-way ANOVA was adopted to compare the mRNA expression based on diet types, postprandial time, and their interaction. The homogeneity test of variance was performed with the Levene test. The mRNA expression based on one diet type in different postprandial point-in-time and the mRNA expression based on one postprandial point-in-time fed with different diet types were analyzed by one-way ANOVA. If there was a significant difference ($P < 0.05$), the mean was sorted using Tukey's multiple range test. Analyses were performed using the SPSS program version 16.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for Windows. All data were presented as means ± S.E.M (standard error of the mean).

RESULTS

Feed intake for different experimental diets

According to Fig. 1, feed intake showed no difference when 18% of fish meal was replaced by rice protein concentrate with microcapsule lysine supplemented ($P = 0.421$). However,
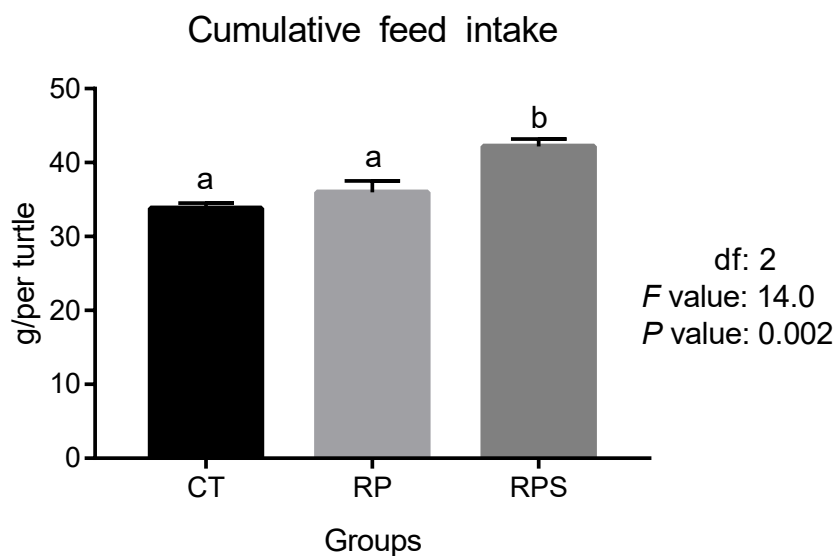
Figure 1 **Feed intake in Chinese soft-shelled turtle fed with different experimental diets.** CT, diets including 60% fishmeal; RP, diets including 42% fishmeal; RPS, diets including 42% fishmeal and 1% squid paste. Values are means of four replications. Means with different lowercase are significantly different ($P < 0.05$).

significantly increased ingestion was observed when 1% of squid paste was included ($P = 0.02$).

Sequential changes upon fasting and effect of refeeding on INSR and LptinR mRNA levels in the brain and intestinal tract

Leptin receptor (LeptinR) mRNA expression in either brain or intestinal tract was affected by the time ($P < 0.001$) and diets ($P = 0.001$, $P = 0.026$) (Figs. 2A and 2B). During fasting in all the depots studied, LeptinR mRNA levels significantly ($P < 0.05$) raised at 3 h fasting and restored to preprandial level at 6–12 h fasting both in the brain and intestinal tract ($P < 0.001$). As for each depot studied, significant differences were observed at F, 3 h, 12 h and 24 h in the brain ($P = 0.01$, $P = 0.03$, $P = 0.03$, $P = 0.04$), as well as 6 h and 24 h in the intestinal tract ($P = 0.005$, $P = 0.038$). Besides, the relative mRNA expression of LeptinR in the brain and intestine was significantly affected by the interaction of diets and time ($P = 0.013$, $P = 0.001$). As regards to the insulin receptor (INSR) (Figs. 2C and 2D), temporal changes of INSR mRNA expression in the brain and intestine reached a peak at 3 h and then restored to a relatively stable level at 6 h ($P < 0.001$). Upon each depot evaluated, significant changes were only found at 3 h fasting in the brain and 6 h fasting in the intestinal tract ($P = 0.011$, $P = 0.019$). Besides, INSR expression was significantly affected by diets ($P = 0.038$, $P = 0.002$) and time ($P < 0.001$) while the interaction ($P < 0.01$) of diets and time was only found in the brain ($P = 0.001$).
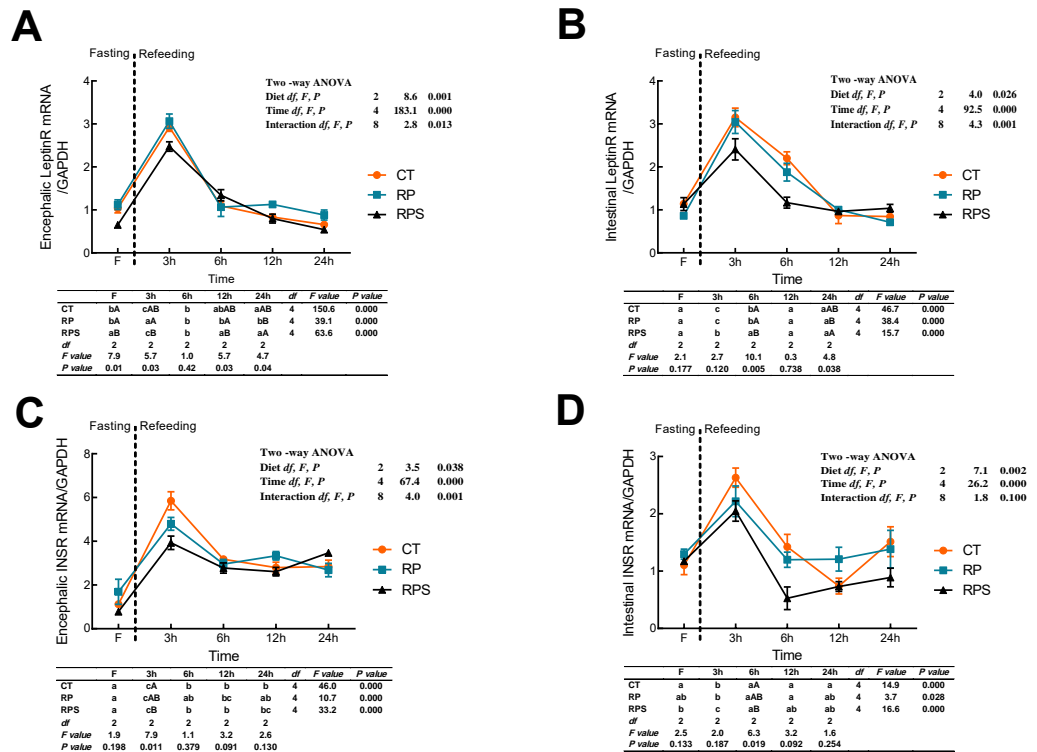
Figure 2 **Sequential changes upon fasting and effect of refeeding on INSR and LeptinR mRNA levels in the brain and intestinal tract.** (A) The relative mRNA expression of encephalic LeptinR; (B) The relative mRNA expression of intestinal LeptinR; (C) The relative mRNA expression of encephalic INSR; (D) The relative mRNA expression of intestinal INSR. Error bars represent mean ± S.E.M. Different lowercase letters indicate significant differences ($P < 0.05$) at different time points within each treatment, whereas different capital letters indicate significant differences ($P < 0.05$) among these three treatments at each sampling point. CT, diets including 60% fishmeal; RP, diets including 42% fishmeal; RPS, diets including 42% fishmeal and 1% squid paste; LeptinR, leptin receptor; INSR, insulin receptor; GAPDH, Glyceraldehydes-3-phosphate dehydrogenase; ns $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Sequential changes upon fasting and effect of refeeding on NPY, POMC and CART mRNA levels in the brain

According to Fig. 3A, the relative expression of NPY mRNA in the brain was significantly affected by the diets ($P < 0.001$) and time ($P < 0.001$). With the time-course of fasting, NPY mRNA levels showed a marked reduction at 3 h ($P < 0.05$), and then followed a significant increase as the peaks were observed at 12 h fasting ($P \leq 0.001$). In terms of each depot, NPY expression in RPS was higher than that in CT and RP group, even though the significant difference was only found at 3 h, 12 h and 24 h fasting ($P = 0.002$, $P = 0.012$, $P = 0.001$). As shown in Figs. 3B and 3C, dynamic POMC and CART mRNA levels followed a similar tendency with LeptinR and INSR. Contrary to the pattern of NPY, CART expression in RPS was lower than that in CT and RP group, and even a significant difference was only found at 24 h fasting ($P = 0.026$). Furthermore, POMC was significantly affected ($P < 0.001$, $P < 0.01$) by time and the interaction of diets and time ($P < 0.001$, $P = 0.003$), as CART was significantly affected by diets and time ($P < 0.001$).
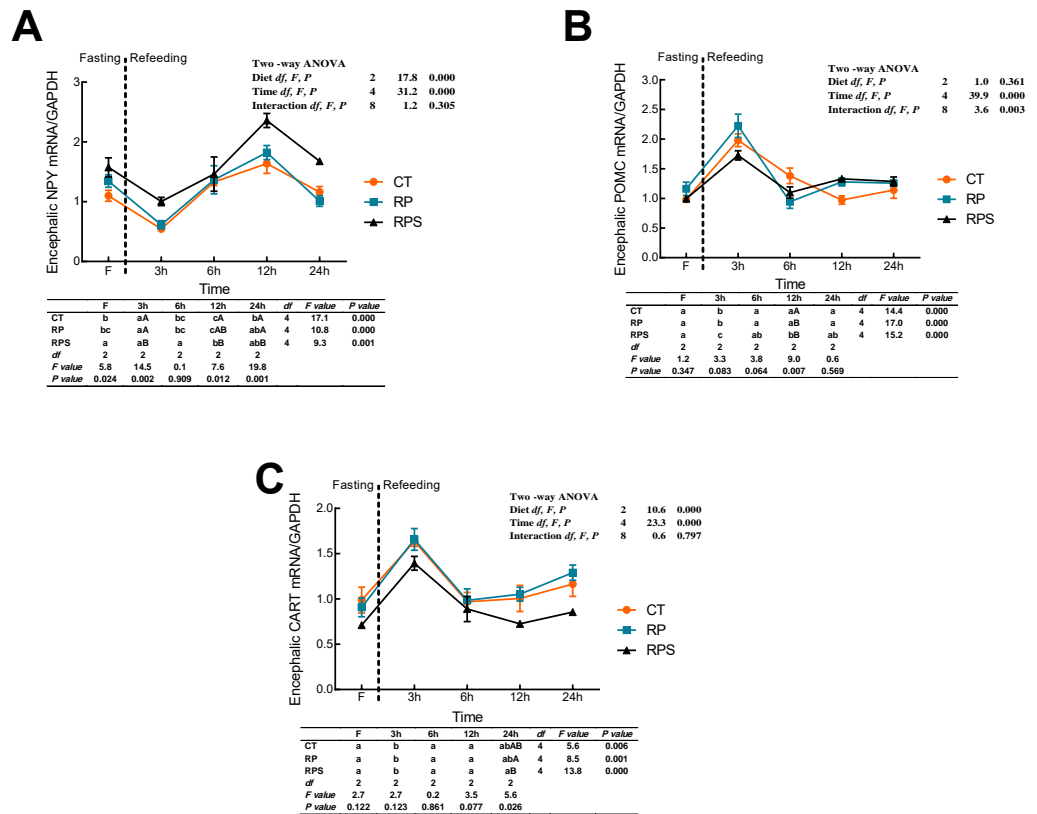
Figure 3 Sequential changes upon fasting and effect of refeeding on NPY, POMC and CART mRNA levels in the brain. (A) The relative mRNA expression of encephalic NPY; (B) The relative mRNA expression of encephalic POMC; (C) The relative mRNA expression of encephalic CART. Error bars represent mean ± S.E.M. Different lowercase letters indicate significant differences ($P < 0.05$) at different time points within each treatment, whereas different capital letters indicate significant differences ($P < 0.05$) among these three treatments at each sampling point. CT, diets including 60% fishmeal; RP, diets including 42% fishmeal; RPS, diets including 42% fishmeal and 1% squid paste; NPY, neuropeptide Y; POMC, pro-opiomelanocortin; CART, cocaine and amphetamine-regulated transcript; GAPDH, Glyceraldehydes-3-phosphate dehydrogenase; ns $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Sequential changes upon fasting and effect of refeeding on CCK and CCK1R mRNA levels in the brain and intestinal tract

CCK and cholecystokinin receptor 1 (CCK1R) mRNA expression were showed in Fig. 4. According to Figs. 4A and 4B, there was a significant increase in CCK and CCK1R mRNA levels of the brain at 3 h postprandial sampling ($P < 0.05$), and continued to drop until 24 h of food deprivation ($P < 0.001$). For each depot studied, the expression of CCK in the brain in RPS reduced significantly at 3 h, 6 h, and 24 h in contrast to that in RP ($P = 0.032$, $P = 0.001$, $P = 0.022$) while significant difference was only found at 3 h fasting in CCK1R expression ($P = 0.016$). In addition, CCK and CCK1R in the brain were significantly affected by diets ($P < 0.001$, $P = 0.001$), time ($P < 0.001$), and the interaction of diets and time ($P = 0.01$, $P = 0.006$). In terms of intestinal CCK mRNA expression (Fig. 4C), intestinal CCK levels increased significantly at 3–6 h fasting and restored to
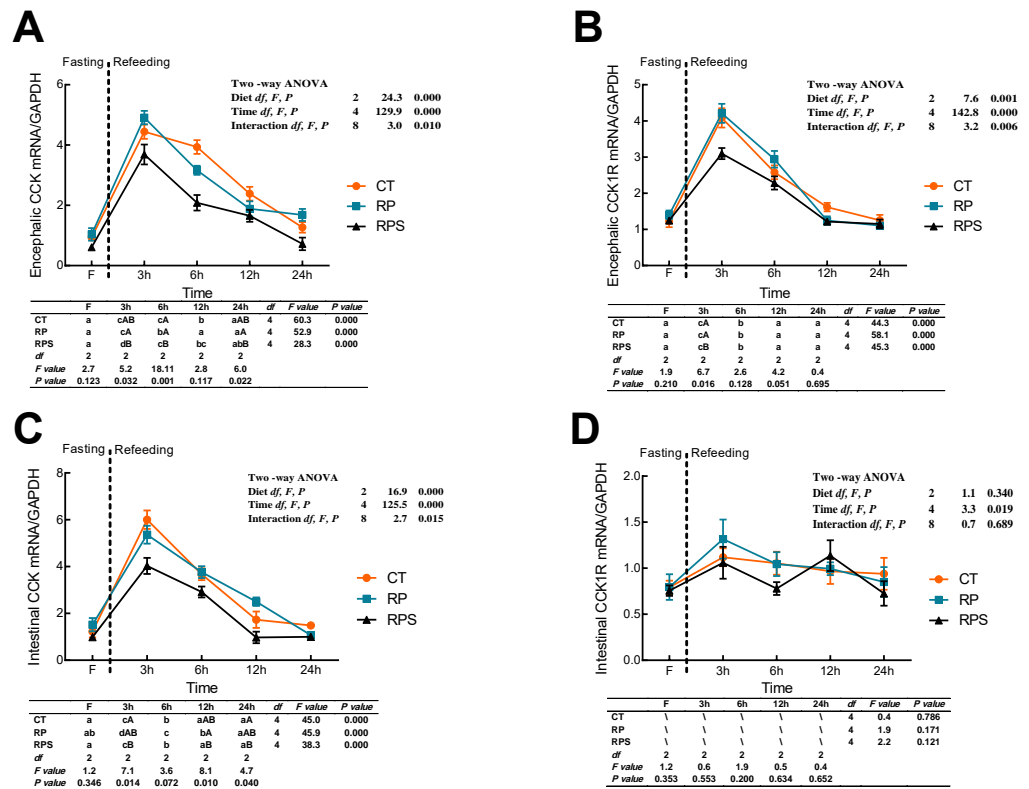
Figure 4 Sequential changes upon fasting and effect of refeeding on CCK and CCK1R mRNA levels in the brain and intestinal tract. (A) The relative mRNA expression of encephalic CCK; (B) The relative mRNA expression of encephalic CCK1R; (C) The relative mRNA expression of intestinal CCK; (D) The relative mRNA expression of intestinal CCK1R. Error bars represent mean ± S.E.M. Different lowercase letters indicate significant differences ($P < 0.05$) at different time points within each treatment, whereas different capital letters indicate significant differences ($P < 0.05$) among these three treatments at each sampling point. CT, diets including 60% fishmeal; RP, diets including 42% fishmeal; RPS, diets including 42% fishmeal and 1% squid paste; CCK, cholecystokinin; CCK1R, cholecystokinin receptor; GAPDH, Glyceraldehydes-3-phosphate dehydrogenase; ns $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

preprandial levels afterward ($P < 0.001$). Additionally, intestinal CCK expression was significantly affected by diets, time, and the interaction of diets and time ($P < 0.001$, $P < 0.001$, $P = 0.015$). As shown in Fig. 4D, CCK1R mRNA levels were only significantly affected by time ($P = 0.019$).

Sequential changes upon fasting and effect of refeeding on GLP1R and PYY mRNA levels in the brain and the intestinal tract

According to Fig. 5A, glucagon-like peptide-1 receptor (GLP1R) in the brain significantly raised its mRNA level at 6 h fasting and then returned to the initial level at 12 h fasting ($P < 0.001$, $P = 0.005$). GLP1R expression in the brain was significantly affected by time ($P < 0.001$), while no statistical difference was found affecting by diets ($P = 0.591$). As with the intestinal CCK1R expression, intestinal GLP1R levels stabilized through the 24 h food deprivation (Fig. 5B). As shown in Figs. 5C and 5D, PYY in both the brain and
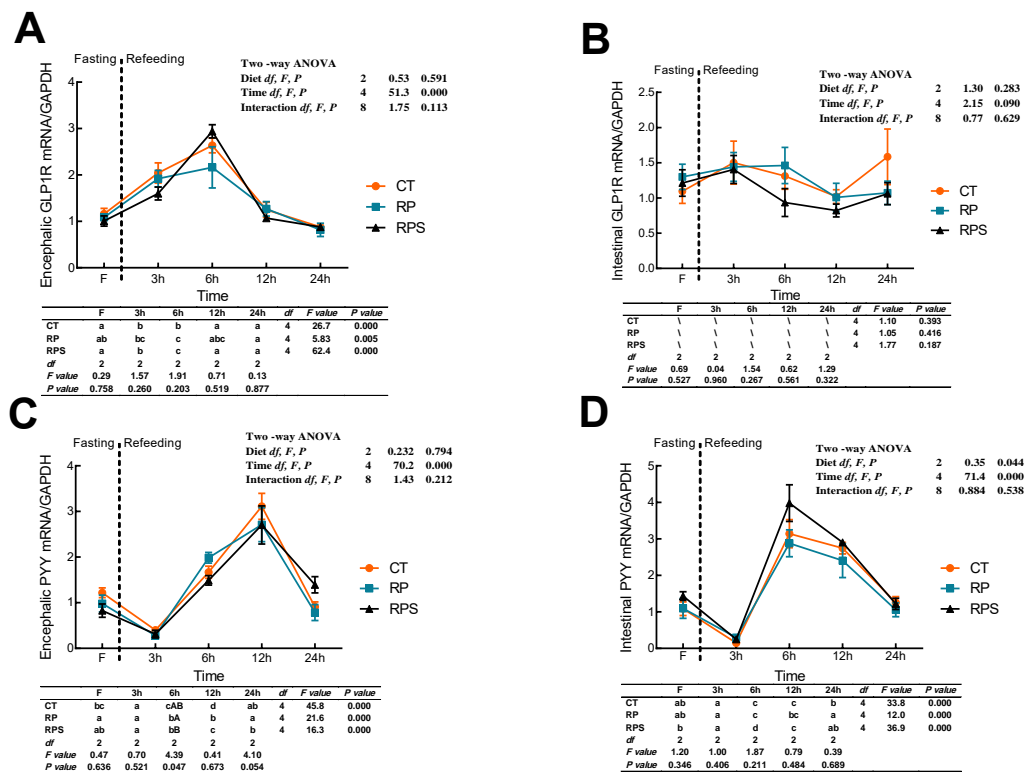
Figure 5 **Sequential changes upon fasting and effect of refeeding on GLP1R and PYY mRNA levels in the brain and intestinal tract.** (A) The relative mRNA expression of encephalic GLP1R; (B) The relative mRNA expression of intestinal GLP1R; (C) The relative mRNA expression of encephalic PYY; (D) The relative mRNA expression of intestinal PYY. Error bars represent mean ± S.E.M. Different lowercase letters indicate significant differences ($P < 0.05$) at different time points within each treatment, whereas different capital letters indicate significant differences ($P < 0.05$) among these three treatments sat each sampling point. CT, diets including 60% fishmeal; RP, diets including 42% fishmeal; RPS, diets including 42% fishmeal and 1% squid paste; GLP1R, glucagon-like peptide-1 receptor; PYY, peptide YY; GAPDH, Glyceraldehydes-3-phosphate dehydrogenase; ns $P > 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

intestine suffered an unexpected reduction, and afterward, a significant increase in their expression with the time-course fasting ($P < 0.001$). The peaks were observed at 12 h fasting in the brain and 6 h fasting in the gut, respectively. Additionally, PYY mRNA levels in the brain were significantly affected by time ($P < 0.001$) as intestinal PYY mRNA levels were significantly affected by diets and time ($P = 0.044$, $P < 0.001$).

DISCUSSION

In the present study, dietary rice protein concentrate inclusion showed no effects on feed intake. The results suggested that rice protein concentrate has excellent palatability to *Pelodiscus sinensis*. Similar results were also found in pacific white shrimp (*Penaeus vannamei*), rainbow trout (*Oncorhynchus mykiss*), and blunt snout bream (*Megalobrama amblycephala*) (*Cai et al., 2018*; *Oujifard et al., 2012*; *Palmegiano et al., 2006*). Nevertheless, squid paste showed a significant attractive effect on *Pelodiscus sinensis*. It might be explained
by the fact that squid paste is rich in many small molecular substances, such as free amino acid, organic acids, and especially the trimethylamine N-oxide (TMAO). These substances have been turned out to be highly useful to the aquatic animal (*Kasumyan & DÖving, 2003*; *Kohbara et al., 2006*; *Tian, 2012*; *Wang et al., 2012*). Although the ingestive behavior may be regulated by the synthetic action of these stimulant substances, the physiological regulatory signals response before and after ingestion is scarce in aquatic animals.

Food deprivation means a critical challenge, which must be quickly and harmoniously addressed by different organs to adjust the negative energy balance (*Palou et al., 2008*). The physiological response to nutritional ingestion during food deprivation provided a comprehensive index of the energy expensed by all activities used to process a diet (*Mccue, 2006*; *Secor, 2009*). After a meal, a series of physiological food intake regulation signals occur in the process of digesting, absorbing, and assimilating ingested nutrients (*Carter et al., 2001*). Thus, the dynamic mRNA expression of the appetitive peptide during short term fasting here could reflect physiological food intake regulation to different nutrition and stimulants.

In the present study, LeptinR and INSR in the brain showed a postprandial increase at 3 h, followed by a significant decrease at 6 h, inferring that LeptinR and INSR in Chinese soft-shelled turtle might induce appetitive peptides directly into the brain to regulate food intake (*Campfield et al., 1995*; *Weigle et al., 1995*). It could be supported by the fact that leptin and insulin have a potently inhibiting effect on food intake (*Billington, 2001*; *Porte, Baskin & Schwartz, 2005*) and transmit their long-acting signal through LeptinR and INSR in the central nervous system (CNS). Similar results also had been observed in mammals (*Baskin et al., 1998*). Intestinal LeptinR and INSR showed a similar tendency with that in the CNS. Nevertheless, compared with CT and RP group, turtles fed the diet with the inclusion of squid paste presented a more abrupt decrease with the time course of 12 h fasting. The diet-induced intestinal LeptinR and INSR expression might suggest the cooperation in the intestine on the regulation of food intake between long- and short-acting anorectic signals. It was supported by the emerging evidence that leptin and insulin receptors are expressed on intestinal L cells, which was regarded as the similar synergism between long and short-term signals in the gut. As for different diet types, a significant increase of LeptinR postprandially was also found when feed intake of grass carp (*Ctenopharyngodon idellus*) decreased (*Huang et al., 2019*). However, the minor effect of leptin was observed in Atlantic salmon (*Salmo salar L.*) fed with different diets at 6 h postprandially, and tilapia (*Oreochromis sp.*) fed with different stimulant at 24 h postprandially (*Sissener et al., 2013*; *Zou et al., 2017*). The conflict might attribute to discrepant sampling time and the effects of leptin in different species.

In the present study, POMC and CART expression followed a similar trend with LeptinR and INSR, with a contrary pattern observed in NPY expression. The results might suggest that the endogenous leptin and insulin in Chinese soft-shelled turtle might regulate short-term food intake through the signals to activate specific efferent pathways (NPY or POMC/CART) like mammals. It was supported by the parallel results in red-bellied piranha (*Pygocentrus nattereri*) (*Volkoff, 2014*), zebrafish (*Danio rerio*) (*Nishio et al., 2012*), Atlantic salmon (*Salmo salar*) (*Valen et al., 2011*) and rats (*Palou et al., 2009*). It could be

further justified by the evidence that anorexigenic POMC and CART are stimulated by leptin (*Schwartz et al., 1997*). At the same time, orexigenic NPY neuron is inhibited, and overlapping signal transduction and transcriptional cascades are activated by insulin (*Hill et al., 2010*; *Spanswick et al., 1997*; *Spanswick et al., 2000*). Additionally, the alteration of NPY and POMC/CART expression in the brain was transient, and their mRNA restored to the preprandial levels at 24 h and 6 h fasting, respectively, inferring that the control power of leptin and insulin is variable to different neuropeptides. NPY in the brain might contribute a more significant long-lasting effect on Chinese soft-shelled turtle. Concerning different diet types, a similar result also found in fish. *Lateolabrax japonicas* fed with palatable diet showed significantly higher mRNA expression of POMC in the hypothalamus than that fed with a control diet at 3 h after feeding (*Liang et al., 2019*). Decreased expression of CART and increased expression of NPY in the brain was observed when a palatable diet was fed in grass carp and tilapia (*Liu et al., 2014*; *Zou et al., 2017*). Therefore, the response of neuropeptides (NPY, CART, and POMC) in *Pelodiscus sinensis* to different diet types might be similar to those in fish.

CCK is produced by I cells in the intestinal mucosa, as well as in the brain and enteric nervous system. CCK interacts with CCK1R and CCK2R expressed in CNS and gastrointestinal tract, in which CCK1R is responsible for ingestion and digestion (*Cummings & Overduin, 2007*). In the present study, CCK expression in both the brain and intestine improved within 6 h post-meal and reverted to the initial level at 12 h fasting time point, which also followed the parallel pattern of LeptinR and INSR. The results indicated that CCK typified a short-acting satiation signal in *P. sinensis*. Similar results were also observed in mammals (*Cummings & Overduin, 2007*; *Kopin et al., 1999*), for example, basal plasma CCK level gradually increase over 10–30 min after meal inhibition and remaining elevated for as long as postprandial 3–5 h in human (*Moran & Kinzig, 2004*). However, a slight change of intestinal CCK1R expression was observed, while CCK1R mRNA levels showed a similar pattern with CCK in the brain. The inconsistent results might indicate that the regulation of food intake by CCK only acted in CNS for this species. In the present study, turtles fed diets included squid paste presented lower peak mRNA level of CCK and CCK1R than that in other groups. It might be accounted for the improved activities of the digestive enzyme in the RPS group (*Sun et al., 2018a*; *Sun et al., 2018b*). Similar results were also found in grass carp (*Liu et al., 2014*; *Huang et al., 2019*). However, only a minor decrease of CCK expression in the brain at 15mins after feeding was found when dietary palatability improved to Cobia (*Rachycentron canadum*) (*Van Nguyen et al., 2013*). The inconsistency might ascribe to the different sampling point-in-time, as warrants further studies.

GLP1 is secreted primarily by L cells in the colon and distal small intestine. Previous studies have asserted that GLP1 could decrease ingestion with the anorectic effects mediated specifically by GLP1R (*Cummings & Overduin, 2007*; *Donahey et al., 1998*; *Verdich et al., 2001*). In the present study, GLP1R expression in the brain ascended after 6 h fasting and continued to decrease with the time course of fasting, but no significant difference was found in intestinal GLP1R expression. Only GLP1R in the CNS followed a similar tendency with LeptinR and INSR, suggesting that GLP1 induced anorexia possibly directed central
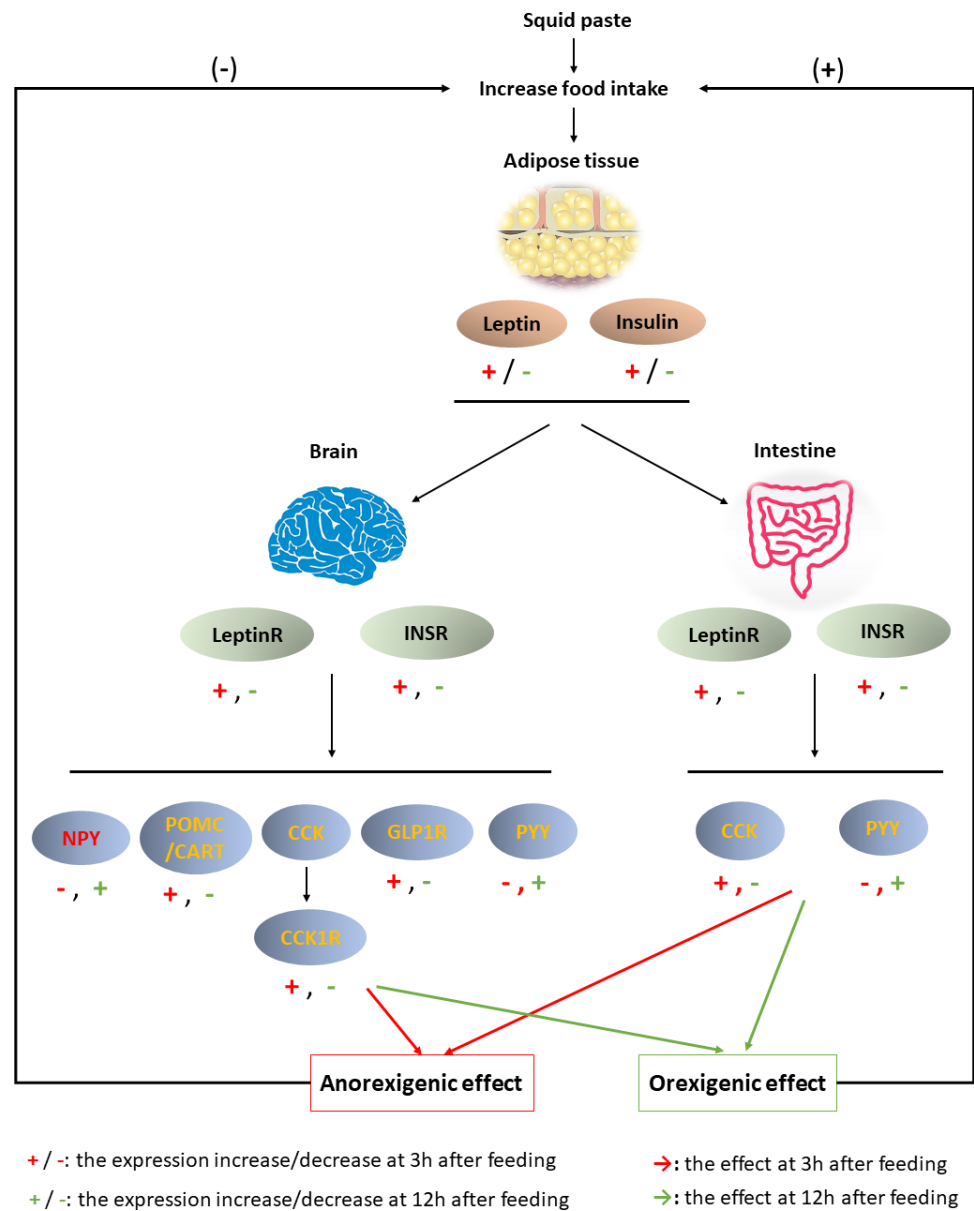
Figure 6   Brain-gut dynamic responses to squid paste during fasting.

pathways. Similar results were also found in mice (*Mus musculus*) (*Baggio et al., 2004*). It was supported by the fact that LeptinR and INSR expressed on L cells augment GLP1 secretion in either gut or hypothalamus (*Anini & Brubaker, 2003*). In addition, peripheral GLP1 could be degraded by DPP-IV in the circulation (*Orskov, Wettergren & Holst, 1993*), which might partly account for the nondiscriminatory GLP1R mRNA level in the intestinal tract. Diet types showed no effects on GLP1R expression in the present study. It might be attributed to that the changed components in the experimental diets were the protein mostly, which showed a negligible effect on stimulating GLP1 secretion compared with

lipids and carbohydrates (*Brubaker & Anini, 2003*; *Smeets et al., 2008*). As with GLP1, PYY is also produced by distal-intestinal L cells. It delays gastric emptying and promotes the ileal brake (*Pironi et al., 1993*). Nevertheless, the present study asserted an inconsistent result in that PYY mRNA expression showed an adverse trend with other gastric satiation peptides, such as CCK, POMC, and CART. The results suggested that PYY in Chinese soft-shelled turtle might not be an anorectic peptide. It could be supported by the research that PYY could activate Y1 and Y2 receptors, which evoke the orexigenic effects through the interactions with each other (*Batterham et al., 2002*). Nevertheless, other reports argued that Y receptors are expressed to medicate NPY-induced feeding, while PYY competitively inhibited the expression of NPY (*Kanatani et al., 2000*). Thus, PYY decreases ingestion by inhibiting NPY neurons through the Y receptor as NPY has more excellent powerful effects on food intake than PYY (*Cummings & Overduin, 2007*). Like GLP1, PYY expression in the brain was not affected by diets. It could be partly explained by the fact that the experimental diets were isoenergetic while this peptide is secreted in proportion to caloric load (*Degen et al., 2005*). However, according to the previous study, postprandial mRNA expression of PYY showed a significant difference in grass carp fed with different diet types (*Huang et al., 2019*). The conflict might ascribe to different experimental species and nutritional composition of diets, Pending further study.

CONCLUSION

In summary, the results obtained here suggested that squid paste is an outstanding stimulant for Chinese soft-shelled turtle. The physiological response to squid paste is shown in Fig. 6. Three hours past feeding, squid paste induced the synthesis of leptin and insulin, which afterward combined with LeptinR and INSR in the brain and intestine, respectively. The anorexigenic peptides, such as POMC, CART, CCK/CCK1R, GLP1R in the brain and CCK in the intestine were activated, while NPY, the orexigenic peptide, was inhibited. Both central and peripheral signals contributed to the anorexigenic effects. Compared with the control group, squid paste led to lower expression of anorexigenic peptides at 3 h past feeding, but higher expression of NPY (orexigenic peptide) at 3 h, 12 h, and 24 h postprandially. These molecular signals in the central and peripheral systems might advance hunger pangs. The changed signals highlight the importance of these peptides and their receptors to short-term food deprivation for this species as well as the effect of squid paste on food intake regulatory mechanism.

Funding

This work is supported by Special Fund for Agro-scientific Research in the Public Interest: the evaluation of new protein sources to replace fish meal in aquafeeds (grant number: 201303053). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:

Agro-scientific Research in the Public Interest: the evaluation of new protein sources to replace fish meal in aquafeeds: 201303053.

Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Cunxin Sun conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Yu Qian performed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Wenbin Liu, Weina Xu and Bo Liu conceived and designed the experiments, prepared figures and/or tables, and approved the final draft.
- Kaizhou Wang performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

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).

Data Availability

The raw measurements are available in a Supplemental File.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.9031#supplemental-information.

REFERENCES

Amaya EA, Davis DA, Rouse DB. 2007. Replacement of fish meal in practical diets for the Pacific white shrimp (*Litopenaeus vannamei*) reared under pond conditions. *Aquaculture* **262**:393–401 DOI 10.1016/j.aquaculture.2006.11.015.

Anini Y, Brubaker PL. 2003. Role of leptin in the regulation of glucagon-like peptide-1 secretion. *Diabetes* **52**:252–259 DOI 10.2337/diabetes.52.2.252.

Baggio LL, Huang Q, Brown TJ, Drucker DJ. 2004. Oxyntomodulin and glucagon-like peptide-1 differentially regulate murine food intake and energy expenditure. *Gastroenterology* **127**:546–558 DOI 10.1053/j.gastro.2004.04.063.

Baskin DG, Seeley RJ, Kuliper J, Lok S, Weigle DS, Erickson JC, Palmiter RD, Schwartz MW. 1998. Increased expression of mRNA for the long form of the leptin receptor in the hypothalamus is associated with leptin hypersensitivity and fasting. *Diabetes* **47**:538–543 DOI 10.2337/diabetes.47.4.538.

Batterham RL, Cowley MA, Small CJ, Herzog H, Cohen MA, Dakin CL, Wren AM, Brynes AE, Low MJ, Ghatei MA. 2002. Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* **418**:650–654 DOI 10.1038/nature00887.

Billington C. 2001. Leptin action in the brain: view from the chair. *International Journal of Obesity* **25**:S53 DOI 10.1038/sj.ijo.0801914.

Brubaker PL, Anini Y. 2003. Direct and indirect mechanisms regulating secretion of glucagon-like peptide-1 and glucagon-like peptide-2. *Canadian Journal of Physiology & Pharmacology* **81**:1005–1012 DOI 10.1139/y03-107.

Cai WC, Jiang GZ, Li XF, Sun CX, Mi HF, Liu SQ, Liu WB. 2018. Effects of complete fish meal replacement by rice protein concentrate with or without lysine supplement on growth performance, muscle development and flesh quality of blunt snout bream (*Megalobrama amblycephala*). *Aquaculture Nutrition* **24(1)**:481–491 DOI 10.1111/anu.12581.

Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. 1995. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* **269**:546–549 DOI 10.1126/science.7624778.

Cao YB, Xue JL, Wu LY, Jiang W, Hu PN, Zhu J. 2011. The detection of 3 leptin receptor isoforms in crucian carp gill and the influence of fasting and hypoxia on their expression. *Domestic Animal Endocrinology* **41**:74–80 DOI 10.1016/j.domaniend.2011.04.002.

Carter C, Houlihan D, Kiessling A, Médale F, Jobling M, Houlihan D, Boujard T, Jobling M. 2001. Physiological effects of feeding. In: *Food intake in fish*. Oxford: Blackwell Science, 297–331.

China Fishery Statistical Yearbook. 2018. *Fishery bureau of ministry of agriculture of the People's Republic of China*. Beijing: China Agriculture Press, 24.

Coutinho F, Peres H, Guerreiro I, Pousãoferreira P, Olivateles A. 2012. Dietary protein requirement of sharpsnout sea bream (*Diplodus puntazzo*, Cetti 1777) juveniles. *Aquaculture* **356**:391–397.

Cowley MA, Smart JL, Rubinstein M, Cerdán MG, Diano S, Horvath TL, Cone RD, Low MJ. 2001. Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature* **411**:480–484 DOI 10.1038/35078085.

Cowley MA, Smith RG, Diano S, Tschöp M, Pronchuk N, Grove KL, Strasburger CJ, Bidlingmaier M, Esterman M, Heiman ML. 2003. The distribution and mechanism of action of ghrelin in the CNS demonstrates a novel hypothalamic circuit regulating energy homeostasis. *Neuron* **37**:649–661 DOI 10.1016/S0896-6273(03)00063-1.

Cummings DE, Overduin J. 2007. Gastrointestinal regulation of food intake. *The Journal of Clinical Investigation* **117**:13–23 DOI 10.1172/JCI30227.

Degen L, Oesch S, Casanova M, Graf S, Ketterer S, Drewe J, Beglinger C. 2005. Effect of peptide YY3-36 on food intake in humans. *Gastroenterology* **129**:1430–1436 DOI 10.1053/j.gastro.2005.09.001.

Donahey JC, Van Dijk G, Woods SC, Seeley RJ. 1998. Intraventricular GLP-1 reduces short-but not long-term food intake or body weight in lean and obese rats. *Brain Research* **779**:75–83 DOI 10.1016/S0006-8993(97)01057-3.

Fan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD. 1997. Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature* **385(6612)**:165–168 DOI 10.1038/385165a0.

Food and Agricultural Organization of the United Nations (FAO). 2014. *State of the world's fisheries and aquaculture 2014.* Rome: FAO Fisheries and Aquaculture Department, 200.

Hevrøy EM, El-Mowafi A, Taylor R, Norberg B, Espe M. 2008. Effects of a high plant protein diet on the somatotropic system and cholecystokinin in Atlantic salmon (*Salmo salar* L.). *Comparative Biochemistry & Physiology Part A Molecular & Integrative Physiology* **151**:621–627 DOI 10.1016/j.cbpa.2008.07.026.

Hill JW, Elias CF, Fukuda M, Williams KW, Berglund ED, Holland WL, Cho Y-R, Chuang J-C, Xu Y, Choi M. 2010. Direct insulin and leptin action on pro-opiomelanocortin neurons is required for normal glucose homeostasis and fertility. *Cell Metabolism* **11**:286–297 DOI 10.1016/j.cmet.2010.03.002.

Hua X, Shui C, He Y, Xing S, Yu N, Zhu Z, Zhao C. 2015. Effects of different feed stimulants on freshwater crayfish (*Procambarus clarkii*), fed diets with or without partial replacement of fish meal by biofeed. *Aquaculture Nutrition* **21**:113–120 DOI 10.1111/anu.12148.

Huang CC, Sun J, Ji H, Oku H, Chang ZG, Tian JJ, Yu EM, Xie J. 2019. Influence of dietary alpha-lipoic acid and lipid level on the growth performance, food intake and gene expression of peripheral appetite regulating factors in juvenile grass carp (*Ctenopharyngodon idellus*). *Aquaculture* **505**:412–422 DOI 10.1016/j.aquaculture.2019.02.054.

Kanatani A, Mashiko S, Murai N, Sugimoto N, Ito J, Fukuroda T, Fukami T, Morin N, Macneil DJ, Lh VDP. 2000. Role of the Y1 receptor in the regulation of neuropeptide Y-mediated feeding: comparison of wild-type, Y1 receptor-deficient, and Y5 receptor-deficient mice. *Endocrinology* **141**:1011–1016 DOI 10.1210/endo.141.3.7387.

Kasumyan AO, DÖving KB. 2003. Taste preferences in fishes. *Fish and Fisheries* **4**:289–347 DOI 10.1046/j.1467-2979.2003.00121.x.

Kohbara J, Miyazaki T, Takii K, Hosokawa H, Ukawa M, Kumai H. 2006. Gustatory responses in Pacific bluefin tuna Thunnus orientalis (Temminck and Schlegel). *Aquaculture Research* **37**:847–854 DOI 10.1111/j.1365-2109.2006.01501.x.

Kopin AS, Mathes WF, McBride EW, Nguyen M, Al-Haider W, Schmitz F, Bonner-Weir S, Kanarek R, Beinborn M. 1999. The cholecystokinin-A receptor mediates inhibition of food intake yet is not essential for the maintenance of body weight. *The Journal of Clinical Investigation* **103**:383–391 DOI 10.1172/JCI4901.

Liang X, Han J, Xue M, Yu H, Huang H, Wu X, Zheng Y, Qin Y, Liang X. 2019. Growth and feed intake regulation responses to anorexia, adaptation and fasting in Japanese seabss, Lateolabrax japonicas when fishmeal is totally replaced by plant protein. *Aquaculture* **498**:528–538 DOI 10.1016/j.aquaculture.2018.09.010.

Liu L, Liang XF, Li J, Yuan X, Zhou Y, He Y. 2014. Feed intake, feed utilization and feeding-related gene expression response to dietary phytic acid for juvenile grass carp (Ctenopharyngodon idellus). *Aquaculture* **424**:201–206.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta}$ method. *Methods* **25**:402–408 DOI 10.1006/meth.2001.1262.

López N, Sánchez J, Picó C, Palou A, Serra F. 2010. Dietary l—leucine supplementation of lactating rats results in a tendency to increase lean/fat ratio associated to lower orexigenic neuropeptide expression in hypothalamus. *Peptides* **31**:1361–1367 DOI 10.1016/j.peptides.2010.03.028.

Mccue M. 2006. Specific dynamic action: a century of investigation. *Comparative Biochemistry & Physiology Part A* **144**:381–394 DOI 10.1016/j.cbpa.2006.03.011.

Mommsen TP. 2000. Glucagon-like peptide1 in fishes: the liver and beyond1. *American Zoologist* **124**:259–268.

Moran TH, Kinzig KP. 2004. Gastrointestinal satiety signals II. Cholecystokinin. *American Journal of Physiology-Gastrointestinal and Liver Physiology* **286**:G183–G188 DOI 10.1152/ajpgi.00434.2003.

National Research Council (NRC). 2011. *Committee on the nutrient requirements of fish and shrimp. Nutrient requirements of fish and shrimp.* Pittsburgh: National Academies Press, 311.

Nishio S-I, Gibert Y, Berekelya L, Bernard L, Brunet F, Guillot E, Le Bail J-C, Sánchez JA, Galzin AM, Triqueneaux G. 2012. Fasting induces CART down-regulation in the zebrafish nervous system in a cannabinoid receptor 1-dependent manner. *Molecular Endocrinology* **26**:1316–1326 DOI 10.1210/me.2011-1180.

Orskov C, Wettergren A, Holst JJ. 1993. Biological effects and metabolic rates of glucagonlike peptide-1 7-36 amide and glucagonlike peptide-1 7-37 in healthy subjects are indistinguishable. *Diabetes* **42**:658–661 DOI 10.2337/diab.42.5.658.

Oujifard A, Seyfabadi J, Abedian Kenari A, Rezaei M. 2015. Growth response and tail-muscle fatty acid quality of Pacific white shrimp, *Litopenaeus vannamei* (Boone) fed with diets containing different levels of rice protein concentrate. *Iranian Journal of Fisheries Sciences* **14**:188–200.

Oujifard A, Seyfabadi J, Kenari AA, Rezaei M. 2012. Growth and apparent digestibility of nutrients, fatty acids and amino acids in Pacific white shrimp, *Litopenaeus vannamei*, fed diets with rice protein concentrate as total and partial replacement of fish meal. *Aquaculture* **342**:56–61.

Palmegiano G, Costanzo M, Dapra F, Gai F, Galletta M, Maricchiolo G, Micale V, Peiretti P, Genovese L. 2007. Rice protein concentrate meal as potential dietary ingredient in practical diets for blackspot seabream (*Pagellus*

bogaraveo). *Journal of Animal Physiology and Animal Nutrition* **91**:235–239 DOI 10.1111/j.1439-0396.2007.00697.x.

Palmegiano G, Daprà F, Forneris G, Gai F, Gasco L, Guo K, Peiretti P, Sicuro B, Zoccarato I. 2006. Rice protein concentrate meal as a potential ingredient in practical diets for rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* **258**:357–367 DOI 10.1016/j.aquaculture.2006.04.011.

Palou M, Priego T, Sanchez J, Villegas E, Rodriguez A, Palou A, Picó C. 2008. Sequential changes in the expression of genes involved in lipid metabolism in adipose tissue and liver in response to fasting. *Pflügers Archiv-European Journal of Physiology* **456**:825–836 DOI 10.1007/s00424-008-0461-1.

Palou M, Sánchez J, Rodríguez AM, Priego T, Picó C, Palou A. 2009. Induction of NPY/AgRP orexigenic peptide expression in rat hypothalamus is an early event in fasting: relationship with circulating leptin, insulin and glucose. *Cellular Physiology and Biochemistry* **23**:115–124 DOI 10.1159/000204100.

Pironi L, Stanghellini V, Miglioli M, Corinaldesi R, De Giorgio R, Ruggeri E, Tosetti C, Poggioli G, Morselli Labate A, Monetti N. 1993. Fat-induced ileal brake in humans: a dose-dependent phenomenon correlated to the plasma levels of peptide YY. *Gastroenterology* **105**:733–733 DOI 10.1016/0016-5085(93)90890-O.

Porte D, Baskin DG, Schwartz MW. 2005. Insulin signaling in the central nervous system a critical role in metabolic homeostasis and disease from *C. elegans* to humans. *Diabetes* **54**:1264–1276 DOI 10.2337/diabetes.54.5.1264.

Powley TL, Phillips RJ. 2004. Gastric satiation is volumetric, intestinal satiation is nutritive. *Physiology & Behavior* **82**:69–74 DOI 10.1016/j.physbeh.2004.04.037.

Riley LG, Fox BK, Breves JP, Kaiya H, Dorough CP, Hirano T, Grau EG. 2008. Absence of effects of short-term fasting on plasma ghrelin and brain expression of ghrelin receptors in the tilapia, Oreochromis mossambicus. *Zoologicalence* **25**:821–827.

Santoso J, Ishizuka Y, Yoshie-Stark Y. 2013. Characteristics of divalent minerals extracted from liver of Japanese common squid Todarodes pacificus under various experimental conditions. *Fisheries Science* **79**:293–301 DOI 10.1007/s12562-012-0584-3.

Schwartz MW, Seeley RJ, Woods SC, Weigle DS, Campfield LA, Burn P, Baskin DG. 1997. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* **46**:2119–2123 DOI 10.2337/diab.46.12.2119.

Secor SM. 2009. Specific dynamic action: a review of the postprandial metabolic response. *Journal of Comparative Physiology B Biochemical Systemic & Environmental Physiology* **179**:1–56 DOI 10.1007/s00360-008-0283-7.

Sissener N, Hemre GI, Espe M, Sanden M, Torstensen B, Hevrøy E. 2013. Effects of plant-based diets on glucose and amino acid metabolism, leptin, ghrelin and GH-IGF system regulation in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition* **19**:399–412 DOI 10.1111/j.1365-2095.2012.00971.x.

Smeets AJ, Soenen S, Luscombe-Marsh ND, Ueland Ø, Westerterp-Plantenga MS. 2008. Energy expenditure, satiety, and plasma ghrelin, glucagon-like peptide 1, and

peptide tyrosine-tyrosine concentrations following a single high-protein lunch. *The Journal of Nutrition* **138**:698–702 DOI 10.1093/jn/138.4.698.

Spanswick D, Smith M, Groppi V, Logan S, Ashford M. 1997. Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature* **390**:521–525 DOI 10.1038/37379.

Spanswick D, Smith M, Mirshamsi S, Routh V, Ashford M. 2000. Insulin activates ATP-sensitive K^+ channels in hypothalamic neurons of lean, but not obese rats. *Nature Neuroscience* **3**:757–758 DOI 10.1038/77660.

Sun CX, Zhang DD, Liu WB, Cai WC, Qian Y, Wang KZ, Li XF, Jiang GZ, Xu WN. 2018b. Growth performance, digestion and metabolism to fish meal replacement by rice protein concentrate in Chinese soft-shelled turtle Pelodiscus sinensis. *Aquaculture* **492**:321–326 DOI 10.1016/j.aquaculture.2018.04.032.

Sun C-X, Xu W-N, Zhang D-D, Li X-F, Li P-F, Jiang G-Z, Liu W-B. 2018a. Different preference is modulated by the feeding stimulant supplementation in different Chinese soft-shelled turtle (*Pelodiscus sinensis*) basic diets. *Aquaculture Nutrition* **24(1)**:195–203 DOI 10.1111/anu.12547.

Tacon AG, Metian M. 2009a. Fishing for feed or fishing for food: increasing global competition for small pelagic forage fish. *AMBIO: A Journal of the Human Environment* **38**:294–302 DOI 10.1579/08-A-574.1.

Tacon AG, Metian M. 2009b. Fishing for aquaculture: non-food use of small pelagic forage fish—a global perspective. *Reviews in Fisheries Science* **17**:305–317 DOI 10.1080/10641260802677074.

Tian A. 2012. *Technology development and industrialization of improved squid paste, South China University of Technology*. Gangzhou: South China University of Technology.

Valassi E, Scacchi M, Cavagnini F. 2008. Neuroendocrine control of food intake. *Nutrition, Metabolism and Cardiovascular Diseases* **18**:158–168 DOI 10.1016/j.numecd.2007.06.004.

Valen R, Jordal A-E, Murashita K, Rønnestad I. 2011. Postprandial effects on appetite-related neuropeptide expression in the brain of Atlantic salmon, Salmo salar. *General and Comparative Endocrinology* **171**:359–366 DOI 10.1016/j.ygcen.2011.02.027.

Van Nguyen M, Jordal AEO, Espe M, Buttle L, Van Lai H, Rønnestad I. 2013. Feed intake and brain neuropeptide Y (NPY) and cholecystokinin (CCK) gene expression in juvenile cobia fed plant-based protein diets with different lysine to arginine ratios. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **165(3)**:328–337 DOI 10.1016/j.cbpa.2013.04.004.

Verdich C, Flint A, Gutzwiller J-P, Naslund E, Beglinger C, Hellstrom P, Long S, Morgan L, Holst J, Astrup A. 2001. A meta-analysis of the effect of glucagon-like peptide-1 (7–36) amide on ad libitum energy intake in humans. *The Journal of Clinical Endocrinology & Metabolism* **86**:4382–4389.

Volkoff H. 2014. Appetite regulating peptides in red-bellied piranha, Pygocentrus nattereri: cloning, tissue distribution and effect of fasting on mRNA expression levels. *Peptides* **56**:116–124 DOI 10.1016/j.peptides.2014.03.022.

Wang CA, Xu QY, Xu H, Yin JS, Wang Y. 2012. Trimethylamine oxidein diets for taimen (*Hucho taimen*): effects on growth performance, muscle composition, gastrointestinal lipase activity and serum biochemical indices. *Chinese Journal of Animal Nutrition* **24(11)**:2279–2286 DOI 10.3969/j.issn.1006-267x.2012.11.029.

Weigle DS, Bukowski T, Foster D, Holderman S, Kramer J, Lasser G, Lofton-Day C, Prunkard D, Raymond C, Kuijper J. 1995. Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. *Journal of Clinical Investigation* **96**:2065–2070 DOI 10.1172/JCI118254.

Zou Q, Huang Y, Cao J, Zhao H, Wang G, Li Y, Pan Q. 2017. Effects of four feeding stimulants in high plant-based diets on feed intake, growth performance, serum biochemical parameters, digestive enzyme activities and appetite-related genes expression of juvenile GIFT tilapia (*Oreochromis* sp.). *Aquaculture Nutrition* **23(5)**:1076–1085 DOI 10.1111/anu.12475.