# Amylin Inhibits the Feeding of the Siberian Sturgeon

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**Research Article**

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# Amylin inhibits the feeding of the Siberian sturgeon

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## Abstract

Amylin is a 37-amino acid polypeptide that has been shown to be involved in feeding regulation in a few mammals, birds and goldfish. To study whether amylin regulates the feeding of Siberian sturgeon, this study cloned amylin of Siberian sturgeon and detected the distribution pattern of amylin in 15 tissues, the expression level in periprandial (pre- and post-feeding), and the changes of food intake and related appetite factors after intraperitonal injection experiment. The results showed that the expression of amylin was highest in hypothalamus, followed by duodenum, telencephalon, forebrain, midbrain, heart and liver, and low in cerebellum, pancreas, valvular intestine and other detection tissues. Compared with 1h pre-feeding, the expression level of amylin in hypothalamus was significantly increased at 1h post-feeding ($P < 0.05$), and the expression level of amylin in duodenum was extremely significantly increased at 1h post-feeding ($P < 0.01$). Compared with the control group (normal saline), intraperitonal injection of 50 ng/g, 100 ng/g and 200 ng/g of amylin significantly inhibited food intake at 1 h ($P < 0.01$), but did not affect food intake at 3h and 6h. The injection of 50ng/g amylin extremely significantly inhibited the cumulative feed intake at 1h, 3h and 6h ($P < 0.01$), and the injection of 100 ng/g amylin extremely significantly inhibited the cumulative feed intake at 1 and 6h ($P < 0.01$), and significantly inhibited the cumulative feed intake at 3h ($P < 0.05$). The injection of 200 ng/g amylin significantly inhibited the cumulative feed intake at 1 and 3h ($P < 0.01$), but did not affect the cumulative feed intake at 6h. After 1h of injection of 50 ng/g amylin, *MC4R* in hypothalamus was significantly increased ($P < 0.05$) and *somatostatin* was extremely significantly increased ($P < 0.01$),

while *amylin* and *NPY* were significantly decreased ($P < 0.05$). *CCK* in valvular intestine was extremely significantly increased ($P < 0.01$), insulin in duodenum was significantly increased ($P < 0.05$), but ghrelin in duodenum had no significant change ($P > 0.05$). These results showed that *Amylin* inhibited feeding in Siberian sturgeon by down-regulating appetite stimulating factor *NPY* and up-regulating appetite suppressing factors *somatostatin*, *MC4R*, *CCK* and *insulin*.

**Keywords** *Amylin*·cloning·feeding·appetite factor·Siberian sturgeon

## 1 Introduction

Ingestion refers to the feeding behavior carried out by the body in order to survive, ensure organ function and obtain energy for various activities. Ingestion is essential for growth and reproduction(Hatef and Unniappan 2019; Okamura et al. 2019). Animal adjusts food intake according to the change of external environmental factors, the change of energy state and appetite factor in the body, which is a complex and precise process(Volkoff 2019). It is well known that hypothalamus is the feeding center, where a series of factors affecting feeding are integrated to regulate feeding behavior (Kim et al. 2020). Some known appetite factors, such as *neuropeptide Y* (*NPY*) (Wei et al. 2014), *melanocortin receptor 4* (*MC4R*) (Li et al. 2019), *cholecystokinin* (*CCK*) (Zhang et al. 2017), *ghrelin* (Zhou et al. 2014), *somatostatin* (Zhu et al. 2017) and *insulin* (Areias and Prada 2015) played an important role in the regulation of feeding. *Amylin* as an appetite factor is one of the hot spots in recent researches.

Amylin, also known as islet amyloid beta protein, was discovered in the pancreas of diabetic patients in 1987, and was named diabetes-associated peptide consisting of 37 amino acids (Cooper et al. 1987). This peptide was later detected in humans and was subsequently renamed amylin (Cooper et al. 1988). Studies on the involvement of amylin in feeding regulation have focused on mammalian rat (Lutz et al. 2018), mouse (Li et al. 2019) and human (Lutz 2012). Studies in chicken (Cline et al. 2010) and Japanese quail (Yuan et al. 2017) have shown that amylin inhibits feeding. Among fish, consumption has been reported only in goldfish (Thavanathan and Volkoff 2006). *Amylin* may inhibit ingestion through the its receptor (Mollet et al. 2004; Reidelberger et al. 2004) and *MC4R*(Li et al. 2019; Roth et al. 2012). *Amylin*'s regulation of food intake may also be related to some appetite factors, such as *NPY*(Morris and Nguyen 2001), *CCK*(Thavanathan and Volkoff 2006) and *Somatostatin* (Luca

et al. 1992).

Although existing studies have shown that *amylin* inhibits feeding, the physiological structures of fish, mammals and birds differ greatly, as well as the diversity of fish species and their living environments, which may lead to different feeding regulation functions and regulatory mechanisms of *amylin*. Siberian sturgeon is an important farmed sturgeon in China, and its caviar is valuable(2010). However, the growth of Siberian sturgeon is slow, and it will take about 10 years for the sturgeon to be cultured as caviar. Further study on the feeding regulation of Siberian sturgeon may accelerate its growth, shorten the reproduction time and improve the quality of caviar. There have been studies on the regulation of feeding by *CCK*(Zhang et al. 2017), *UCN3*(Tang et al. 2019) ,*NPY*(Yuan et al. 2019) and *Spexin* (Tian et al. 2020), but no report was found on the regulation by *amylin* in Siberian sturgeon. In order to study regulation of amylin on the feeding function of Siberian sturgeon, this study cloned amylin from Siberian sturgeon and analyzed its expression pattern. The effects of amylin on feeding regulation of Siberian sturgeon were studied by detecting the periprandial expression of *amylin* and the changes of food intake and appetite factor after intraperitoneal injection of amylin.

## 2 Materials and methods

### 2.1 Laboratory animals, reagents, and medicines

The Siberian sturgeon juvenile used in this experiment was purchased from Sichuan Runzhao Fishery Co., Ltd or Hongya Donglong Sturgeon Industry Technology Co., Ltd. The Siberian sturgeon juvenile was temporarily domesticated in the Aquaculture Laboratory of Sichuan Agricultural University. The water source was aerated tap water, flowing water culture, 12h light /12 dark photophoid, and the water temperature was controlled at (20.1±1.2℃). Fishes were fed to satiety once-daily at 14:00, with commercial sinking pellets (particle size =1.5mm, crude protein ≥49.0%, crude fat ≥10.0%, moisture ≤10.0, crude fiber crude ≤4.0, crude ash ≤11.0%, total phosphorus ≥1.0, lysine ≥2.7%;Tongyi, Suzhou, China). The feeding amount was 3% of body weight, and the residual bait was sucked out with a hose. Fish are acclimated for at least two weeks before formal experiments begin. All animals handling procedures were approved by the Animal Care and Use Committee of Sichuan Agricultural University.

The total RNA extraction kit used in this experiment was provided by Chengdu Fuji

Biotechnology Co., Ltd., and the reverse transcription kit was provided by TAKARA. Amylin polypeptide (purity > 95%) synthesized by Shanghai Botai Biotechnology Co., Ltd., was dissolved in normal saline.

### 2.2 Experimental design and animal sampling

#### 2.2.1 Gene cloning and tissue distribution sampling

Six juvenile Siberian sturgeon (100.6±10.5g, purchased from Sichuan Runzhao Fishery Co., Ltd.) were used for cloning and tissue distribution experiments. Before the experiment, the fish were fasted for 24h, anaesthetized with 0.01% MS-222, then were weighed and decapitated, and the hypothalamus was extracted for cloning of Amylin. Fifteen tissues, including forebrain, midbrain, hypothalamus, cerebellum, medulla oblongata, stomach, pyloric caecum, duodenum, valvular intestine, rectum, heart, liver, pancreas, spleen and Trunk kidney, were collected for tissue distribution analysis.

#### 2.2.2 periprandial experiment

Sixty-three juvenile Siberian sturgeon with similar body weight (100.6±10.5g, purchased from Sichuan Runzhao Fishery Co., Ltd.) were randomly selected and divided into 7 groups with 3 replicates per group and 3 fish per replicate. Groups 1-5 were fasting group, and groups 6 and 7 were feeding group, which were fed at 3% body weight at 2:00 p.m. every day. Six fish from each group were randomly selected for sampling. Samples were collected at -3 h (11:00), -1 h (13:00), 0 h (14:00), +1 h (15:00), and +3 h (17:00) in fasting group, and at +1 h and +3 h in feeding group, respectively. Samples of hypothalamus and duodenum were frozen in liquid nitrogen and stored at -80℃.

#### 2.2.3 Injection experiment

36 healthy Siberian sturgeon juvenile (body weight 89. 2 ±16.4 g, and Purchased from Hongya Donglong Acipenser Industry Technology Co., Ltd.) were randomly selected and divided into 4 groups with 3 replicates per group and 3 fish per replicate. 30 mins before feed, fish were intraperitoneally injected with 0 ng/g (normal saline)BW, 50ng /g BW, 100ng /g BW and 200ng /g BW of amylin, then measured the amount of food consumption after 1 h, 3 h and 6 h of injection.

According to the results of food intake, 18 healthy juvenile Siberian sturgeon (body weight 71.6±7.7g, purchased from Hongya Donglong Acipenser Industry Technology Co., Ltd.) were selected and divided into two groups with 3 replicates per group and 3 fish per replicate. 30 min before feed, they were intraperitonally injected with 0 ng/g BW (saline) and 50 ng/g of amylin, respectively. Food intake was measured 1 h after injection, and the hypothalamus, duodenum and valvum were collected for analysis the expression levels of amylin and appetite factor (*CCK*, *insulin*, *amylin*, *NPY*, *somatostatin*, *ghrelin* and *MC4R*).

### 2.3 Gene cloning and sequence analysis

Total hypothalamic RNA of Siberian sturgeons was extracted according to the manual of TAKARA RNAISO Plus Reagent. The concentration and integrity of RNA were determined by nucleic acid analyzer and 1.0% agarose gel electrophoresis. RNA was reverse transcribed into cDNA according to the instructions of TAKARA reverse transcription kit (article No. RR037A). Primer5 was used to design *amylin* primers based on the nucleotide sequence of *amylin* from Siberian sturgeon in the transcriptome, and the related primers were all synthesized by Sangon Bioengineering (Shanghai) Co., Ltd. (primer sequences are shown in Table 1). PCR amplification system was consisted 5 μL PCR Mastermix, 1 μL cDNA template, 0.5 μL upstream and downstream primers, as well as 4 μL water. The PCR reaction procedure was as follows: pre-denaturation at 94℃ for 5 mins;94℃ denaturation 30s, specific annealing temperature 55℃ annealing 45s, 72℃ 90s extension, 34 cycles;72℃ extended for 7mins, 4℃ permanent. PCR products with concentration of 1.5% agarose gel electrophoresis, using gel imaging system detects amplification bands, use TIANGEN Midi DNA Purification Kit stripe Purification purposes bands, and cloned into pMD19-T carrier, import DH5 alpha cells after heat shock and join the LB medium recovery, recovery of bacteria liquid was apply LB solid culture medium for culture, pick a single colony in the LB liquid culture medium to expand training, bacteria liquid by sangon biological engineering (Shanghai) co., LTD. Sequencing. The deduced amino acid sequences of Amylin from Siberian sturgeon were compared with those of other Amylin species in GenBank.

### 2.4 Real-time fluorescence quantitative detection of amylin and appetite factor

*CCK*, *insulin*, *Amylin*, *NPY*, *somatostatin*, *ghrelin*, and *MC4R* mRNAs were amplified by

real-time PCR using Bio-Rad CFX96 Real-time PCR assay system (Bio-Rad, Hercules, CA, USA) and SYBR Premix EX TMII(Takara, China Dalian) according to the instructions. Primer Premer 5.0 was used to design the qPCR primer sequences for all genes (Primer sequences are shown in Table 1). The amplification efficiency of the primers was greater than 91%, and the correlation coefficient (R2) of the primers was greater than 0.995. The qPCR reaction conditions were as follows: heating to 95℃ for 5 min, DNA denaturation at 95℃ for 15 s, annealing at 61℃ for 30s, extension at 72℃ for 30s, 40 cycles.Template control (NTC) was not used for negative control. To quantify gene expression, reference genes were used to normalize target genes and relative CT was used to compare expression levels.

Table 1 List of clonal primers and qPCR primers

| Primer name | Primer sequence(5′–3′) |
|---|---|
| *amylin* -F | GAAGTGTACAATGTGTTACTTGAG |
| *amylin* -R | ATGACTACGCCGTGTCCTAA |
| *amylin* -qF | ACCACGGCAAACAGGGA |
| *amylin*-qR | TGGATCGGACGAGGAAGT |
| *cck*-qF | GAGGGTAGTCCTGTAGCATCTGA |
| *cck*-qR | TTCTACCAGACGAGCCTTTCC |
| *npy*-qF | GCTGGCTACCGTGGCTTTC |
| *npy*-qR | GACTGGACCTCTTCCCATACCT |
| *Insulin*-qF | GTTGCTTCATTGGAAACTCG |
| *Insulin*-qR | TGGTCTGTGGAGAAAGGGG |
| *somatostatin*-qF | GCAGAACTGTTGTCCGAGC |
| *somatostatin*-qR | CAGGGTTGCCATTAGCG |
| *MC4R*-qF | ATGAAGAGAATCGCAGTCCT |
| *MC4R*-qR | GGTGGAGAAAGAATGGTGC |
| *ghrelin*-qF | CCAAGGTGACACGTCGAGATTC |
| *ghrelin*-qR | TCCTGATACTGAGATTCTGACATTGAG |
| $\beta$*-actin*-qF | GTTGGTATGGGACAGAAGGACA |
| $\beta$-actin-qR | CCAGTTGGTAACAATGCCGT |

### 2.5 Statistical analysis

All experimental data were expressed as Mean ± SEM. Results were processed by SPSS 22.0 (SPSS Sinc., Chicago, IL, USA). After homogeneity test of variance, one-way ANOVA analysis or t-test was used to analyze the data. If $P < 0.05$, it would be marked as significant difference, and if $P < 0.01$, it would be marked as extremely significant difference.

## 3 Results

### 3.1 Cloning and sequence analysis of Amylin

The full-length CDS of Amylin obtained in this study was 387 bp, encoding 128 amino acids. The first 21 amino acids of the presumed amino acid sequence were signal peptides, and there was a 37-amino acid Amylin polypeptide between the C-terminal KR and GKR cleaved sites (Figure 1). Further analysis of amino acid sequences of different species revealed that functional peptides of Amylin of different species were relatively conservative, with cysteine at amino acid sites No. 2 and No. 7 (Figure 2). The phylogenetic tree analysis showed that the Amylin of the Siberian Acipenser was clustered into a single branch with the gallus (Fig. 3). Similarity analysis showed that the similarity between Siberian sturgeon and carp, Wolf, human, mouse, Pseudonaja textilis, Osmerus mordax, rat, Xenopus laevis and gallus was 40.5%, 58.4%, 56.2%, 50.5%, 57.8%, 50%, 48.4%, 38% and 50.8%, respectively, and Amylin of the Siberian sturgeon is most similar to Wolf and least similar to Xenopus laevis.

Fig. 1 Amylin nucleotide sequence and presumed amino acid sequence of Siberian sturgeon. The gray area is the signal peptide, and the underlined area is the functional peptide of amylin.

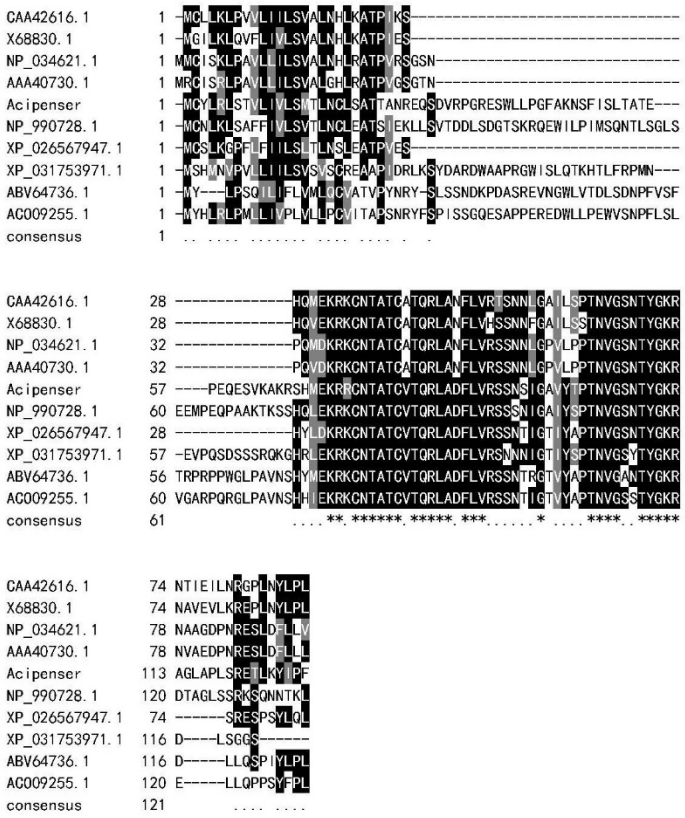

Fig. 2 Reweight comparison of amino acid sequences of Amylin from different species. Species name and GenBank accession number: CAA42616.1 Canis lupus familiaris，X68830.1 Homo sapiens，NP_034621.1 Mus musculus，NP_990728.1 precursor Gallus gallus，Acipenser Baerii，XP_ 026567947.1e Pseudonaja textilis，XP_ 031753971.1 Xenopus tropicalis，ABV64736.1 Carassius

auratus，ACO09255.1 Osmerus mordax，AAA40730.1 Rattus norvegicus. The different colors of amino acids indicate the differences in amylin amino acids between different species.

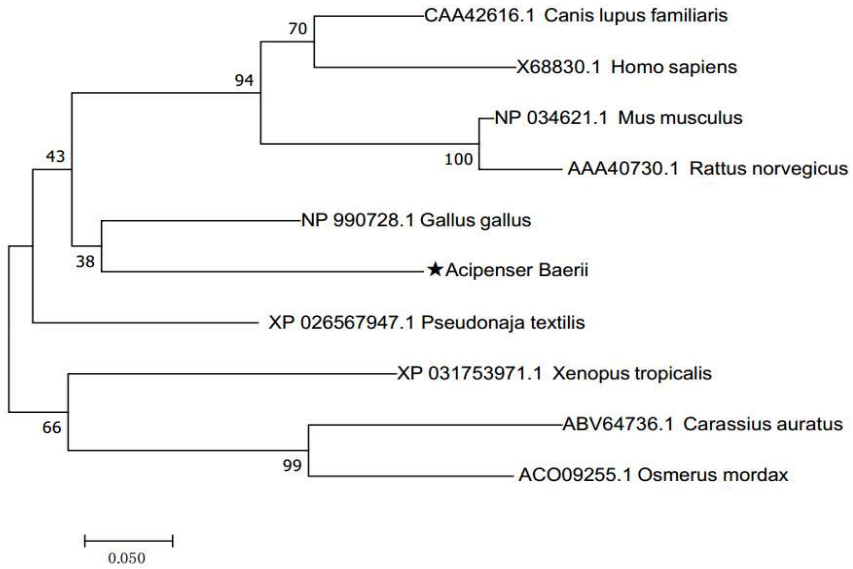


Fig. 3 Evolutionary tree analysis of Amylin amino acids of different species. Species name and GenBank accession number: CAA42616.1 Canis lupus familiaris，X68830.1 Homo sapiens，NP_034621.1 Mus musculus，NP_990728.1 precursor Gallus gallus，Acipenser Baerii，XP_026567947.1e Pseudonaja textilis，XP_ 031753971.1 Xenopus tropicalis，ABV64736.1 Carassius auratus，ACO09255.1 Osmerus mordax，AAA40730.1 Rattus norvegicus.

### 3.2 Tissue distribution of Amylin

Among the 15 tissues tested in this experiment, the expression of amylin was the highest in hypothalamus, followed by duodenum, the higher in the forebrain, Medulla oblongata, mesencephalon ，heart and liver, and the lower in cerebellum, pancreas, valvular intestine and other detected tissues (Figure 4).

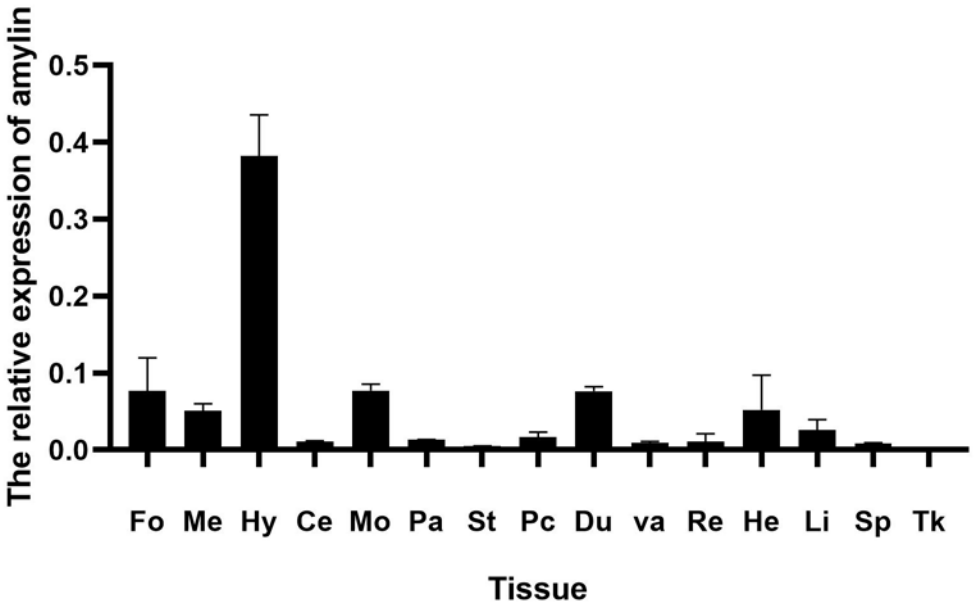


Fig. 4 Tissue expression pattern of Amylin of Siberian sturgeon. Xsl-fo, forebrain; Me, the midbrain; HY, hypothalamus; Ce, the cerebellum; Mo, the medulla; Pa, pancreatic; St, the stomach; PC, pyloric caecum; Du, duodenum; Im va, valvular intestine; Re, rectum; He, the heart; Li, liver; Sp, spleen; Tk, kidney. Data are expressed as mean ±SEM (n = 6).

### 3.3 Changes in Amylin expression of Siberian sturgeon at periprandial

At +1h, compared with fasting group, the expression of amylin in hypothalamus and duodenum of feeding group was significantly increased ($P < 0.05$), and the expression of amylin in duodenum was extremely significantly increased ($P < 0.01$). At +3h, compared with fasting group, there was no significant change in the expression of amylin in hypothalamus and duodenum of feeding group ($P > 0.05$). The expression level of amylin at -3h in fasting group was significantly lower than that at -1h ($P < 0.05$), and there was no significant difference among other groups ($P > 0.05$) (Fig. 5 and 6).

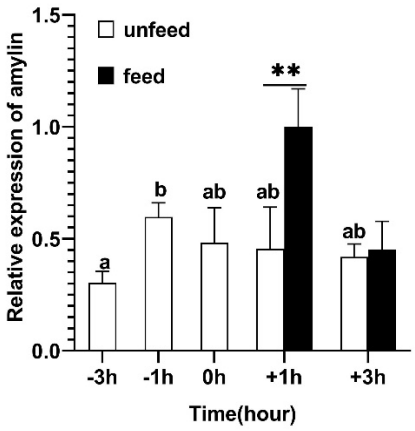


Fig. 5 Expression of Amylin in the hypothalamus of Siberian sturgeon at periprandial. Data were expressed as mean ±SEM (n = 6) *, representing significant P < 0.05, and **, representing extremely significant P < 0.01.

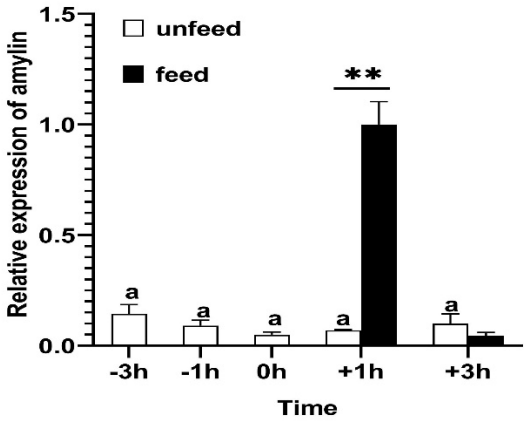


Figure 6 Amylin expression in duodenum of Siberian sturgeon at periprandial. Data were expressed as mean ±SEM (n = 6) *, representing significant P < 0.05, and **, representing extremely significant P < 0.01.

### 3.4 Influence of Amylin injection on feed intake and appetite factor of Siberian sturgeon

Compared with the control group (0 ng/g), intraperitonal injection of 50 ng/g, 100 ng/g and 200 ng/g of amylin significantly inhibited food intake at 1 h (P < 0.01), but did not affect food intake at 3h and 6h (Fig. 7). The injection of 50ng/g amylin significantly inhibited the cumulative feed intake at 1h, 3h and 6h (P < 0.01). The injection of 100 ng/g amylin significantly inhibited the cumulative feed intake at 1 and 6h (P < 0.01), and significantly inhibited the cumulative feed intake at 3h (P < 0.05). (Fig. 8). The injection of 200 ng/g amylin significantly inhibited the cumulative feed intake at 1 and 3h (P < 0.01), but did not affect the cumulative feed intake at 6h (Fig. 8).

Compared with the control group, *MC4R* in hypothalamus was significantly increased ($P < 0.05$), *somatostatin* was significantly increased ($P < 0.01$), and *amylin* and *NPY* were significantly decreased ($P < 0.05$) after injection of 50 ng/g amylin 1h. *CCK* in valvular intestine was significantly increased ($P < 0.01$), *insulin* in duodenum was significantly increased ($P < 0.05$), but *ghrelin* in duodenum was not significantly changed ($P > 0.05$) (Figure 9).

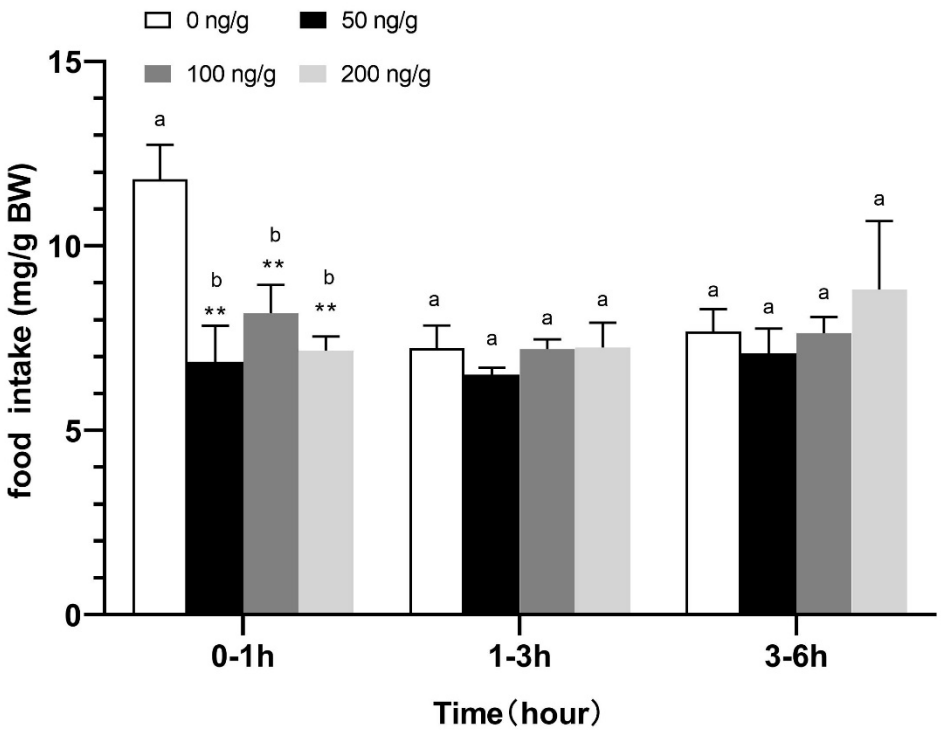


Fig. 7 Effects of intraperitoneal injection of amylin on feed intake of Siberian sturgeon. Food intake was calculated at 1, 3, and 6h after injection. Data are expressed as mean ±SEM (n = 6). * represents significant difference $P < 0.05$, and ** represents extremely significant difference $P < 0.01$.

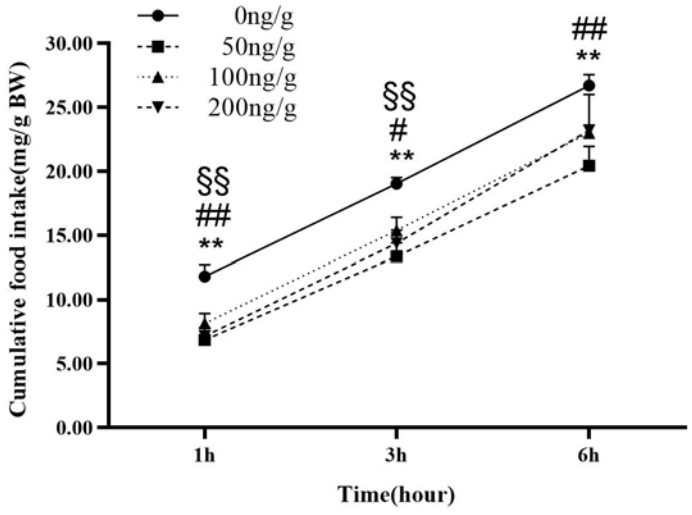

Fig. 8 Effects of intraperitoneal injection of amylin on cumulative feed intake of Siberian sturgeon.* indicates significant difference between 50ng/g BW and normal saline control group;# indicates significant difference between 100ng/g BW and normal saline control group; § indicates that there is a significant difference between 200ng/g BW and normal saline control group.

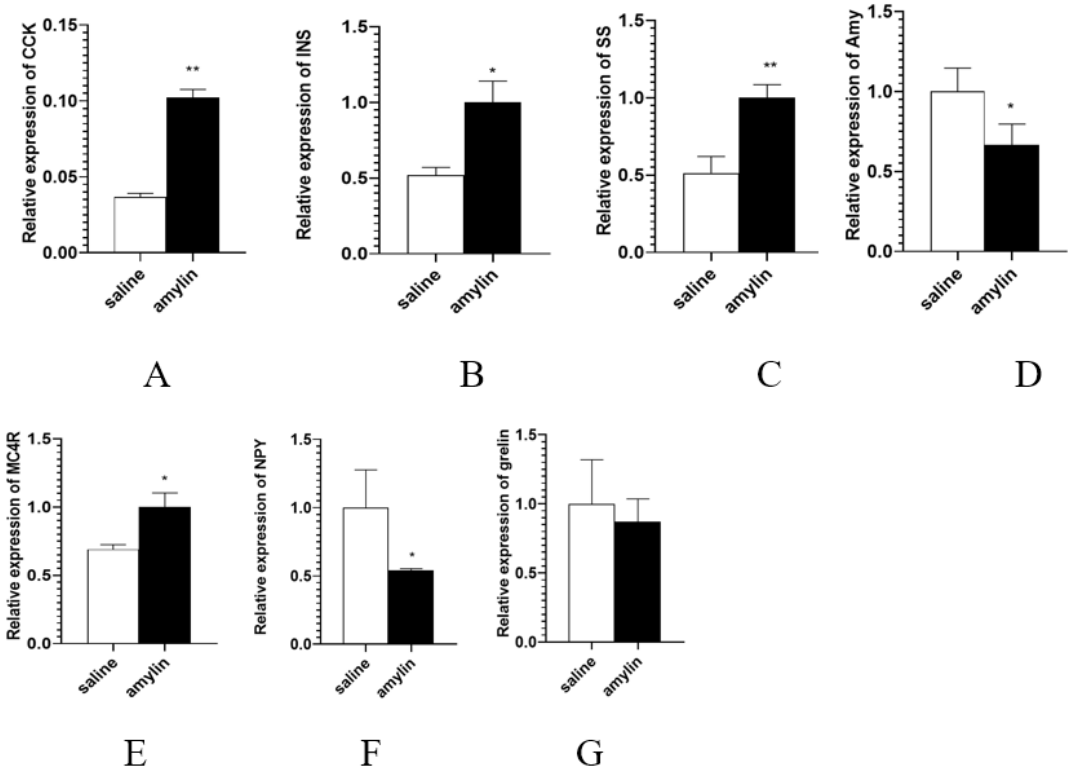


Fig. 9 Effect of intraperitoneal injection of amylin on the expression of appetite factor of Siberian sturgeon 1 h later. The expression levels of CCK (A), insulin (B), somatostatin (C), amylin (D), MC4R (E), NPY (F) and ghrelin (G) were determined after 100 ng/g of amylin.Data are expressed as mean ±SEM (n = 6).* represents significant difference $P < 0.05$, and ** represents extremely significant difference $P < 0.01$.

## 4. Discussion

### 4.1 Molecular characteristics and tissue distribution pattern of amylin

In this study, we cloned *amylin* from the Siberian sturgeon for the first time, with a full-length CDS of 387 bp, and analyzed the amino acid sequences of precursor *amylin* from different species. Amylin has previously been identified in rat (Leffert et al. 1989), zebrafish (Westermark et al. 2002),

puffer fish (Chang et al. 2004) and goldfish (R. et al. 2008). To our knowledge, this is the first time that *amylin* has been cloned from a sturgeon family. Like most species, *amylin* has a 37-amino acid amylin polypeptide between the C-terminal KR and GKR cleavage sites, and a cysteine at sites 2 and 7, which can form disulfide bonds between them, which is necessary for *amylin* to function (Roberts et al. 1989). The phylogenetic tree analysis showed that the *amylin* of the Siberian Acipenser was clustered into a clade with gallus, which is closer to mammals and birds than to amphibians and reptiles, which may be related to the special evolutionary status of sturgeon (Du et al. 2020).

Amylin was highly expressed in the brain region of the Siberian sturgeon, especially in the hypothalamus, and in the duodenum of the periphery. Studies in rats (Skofitseh et al. 1995), chickens (Fan et al.) and goldfish (Martínez-álvarez et al. 2008) have also shown that *amylin* is highly expressed in the brain. However, unlike the Siberian sturgeon, the expression level in the hindbrain of the rats was the highest, which might be caused by the species difference. The high expression of amylin in the hypothalamus and duodenum of the Siberian sturgeon suggests that amylin may be involved in feeding regulation.

### 4.2 Amylin regulates feeding

Appetite factor changes significantly periprandial in response to ingestion. In this study, it was found that *amylin* in the hypothalamus and duodenum increased post-feeding significantly, which was consistent with the results in rats (Alam et al. 1992). The identified anorexia factors, such as *Urocortin-3* (Zhang et al. 2016), *CCK*(Zhang et al. 2017) and *Spexin* (Tian et al. 2020), also increased significantly post- feeding.These results suggest that amylin may suppress appetite in Siberian sturgeon.

In order to verify the inhibitory effect of amylin on feeding in Siberian sturgeon, intraperitoneal injection experiment was carried out in this study. The results showed that the intraperitoneal injection of 50 ng/g, 100 ng/g and 200 ng/g of amylin significantly inhibited food intake at 1 h ($P < 0.01$), but did not affect food intake at 3h and 6h. The injection of 50ng/g amylin significantly inhibited the cumulative feed intake at 1h, 3h and 6h ($P < 0.01$), and the injection of 100 ng/g amylin significantly inhibited the cumulative feed intake at 1 and 6h ($P < 0.01$), and significantly inhibited the cumulative feed intake at 3h ($P < 0.05$). The injection of 200 ng/g amylin significantly inhibited the cumulative

feed intake at 1 and 3h ($P < 0.01$), but did not affect the cumulative feed intake at 6h. It can be seen that amylin inhibitis feeding in Siberian sturgeon. Similar results have been reported in goldfish (Thavanathan and Volkoff 2006), Japanese quail (Yuan et al. 2017), and rats (Reiner et al. 2017).

### 4.3 Relationship between Amylin and other appetite factors

The role of appetite factors in regulating food intake is often not isolated, and it will cause the changes of other appetite factors, and then regulate food intake through the integration of feeding center (Helene 2016). The results of this study showed that after intraperitoneal injection of amylin(50 ng/g BW), the mRNA expression levels of NPY in the hypothalamus were significantly decreased, while the mRNA expression levels of somatostatin and MC4R were increased, also the mRNA expression levels of CCK in valvular intestine and insulin in duodenum were increased. However the mRNA expression levels of ghrelin in duodenum were not significantly changed.

Previous studies in our laboratory have shown that *NPY* can inhibit the expression of *MC4R* in Siberian sturgeon, thereby promoting feeding (Yuan et al. 2019). *NPY* can promote feeding in rats, while amylin can counteract the appetite-stimulating effect of *NPY*, and it was observed in the paravicular nucleus that amylin can reduce the level of *NPY* (Morris and Nguyen 2001) which is similar to the results of this study. It is speculated that amylin may inhibit the feeding of Siberian sturgeon through *NPY*. In this study, the expression of *MC4R*, an appetite suppressant, in the hypothalamus was found to be increased after injection of amylin. Similarly, it has been observed in mice that amylin can increase the protein level of MC4R, thus inhibiting feeding (Li et al. 2019). Amylin in Siberian sturgeon may promote the expression of *MC4R* by inhibiting the expression of *NPY*, and finally achieve the effect of inhibiting feeding.

In this study, amylin promoted the expression of *somatostatin*. Similar rat amylin promotes the secretion of *somatostatin* in the stomach (Zaki et al. 2002), and the peripheral infusion of *somatostatin* reduces the concentration of amylin in rats (Luca et al. 1992). It can be seen that the two hormones secreted by the pancreas may interact with each other.

Insulin and amylin are both secreted by pancreatic beta cells, and there is a functional interaction between them(Ratha et al. 2019). *Insulin* is an appetite suppressant, and its expression level was observed to be inhibited after the injection of amylin in this study, suggesting that *amylin* may play a

regulatory role in feeding by inhibiting *insulin* secretion, thereby causing changes in *Insulin* receptor substrates, etc. (Lv et al. 2019).

In this study, *amylin* of the Siberian sturgeon had a higher distribution in the brain and duodenum, which was similar to the distribution of *CCK* (Zhang et al. 2017). In this study, it was found that amylin could promote the expression of *CCK*. Injection of 1ng/g of amylin and CCK into the ventricle of goldfish did not affect feeding, but combined injection of 1ng/g of amylin and CCK inhibited feeding (Thavanathan and Volkoff 2006). These results suggested that amylin could cooperate with CCK to inhibit the feeding of Siberian sturgeon.

In this study, after the injection of amylin, the expression of *ghrelin* in the duodenum was not affected, and *ghrelin* did not affect the feeding regulation of amylin (Osto et al. 2007), and *amylin* may not regulate food intake through *ghrelin*.

These results suggested that *amylin* could inhibit feeding by down-regulating the expression of appetite stimulating factor *NPY* and up-regulating the expression of appetite suppressing factors, such as *somatostatin*, *MC4R*, *CCK* and *insulin*.

## 5 conclusion

In this study, *amylin* was cloned from Siberian sturgeons with a full-length CDS of 387 bp, and its tissue expression pattern was analyzed. The highest expression of *amylin* was found in hypothalamus, followed by duodenum, telencephalon, forebrain, midbrain, heart and liver, and low expression in cerebellum, pancreas, valvular intestine and other tissues detected. In the Siberian sturgeon, *amylin* inhibited feeding by down-regulating appetite stimulating factor *NPY* and up-regulating appetite suppressing factors *somatostatin*, *MC4R*, *CCK* and *insulin*. These results provide a theoretical basis for studying the mechanism of feeding and growth of Siberian sturgeon.

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**Declarations**

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**Compliance with ethical standards:** This study was complied with the ethical statements. All experiments were approved by the Animal Care and Use Committee of Sichuan Agricultural University and followed the guidelines for animal experiments of Sichuan Agricultural University (No.DKY-S20150812).

**Availability of data and material：** We ensure that all the data are true and reliable.

**Conflict of interest:** The authors declare that they have no conflict of interest.

**Authors' contributions：** All the authors were involved in the design or writing of this article. Mei Wang and Shaoqi Xu paid a similara mount of work and carried out experiments and thesis writing. Zhiqiong Li is corresponding author.

**Code availability：** SPSS 22.0（SPSS Inc., Chicago, IL, USA）

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# Figures

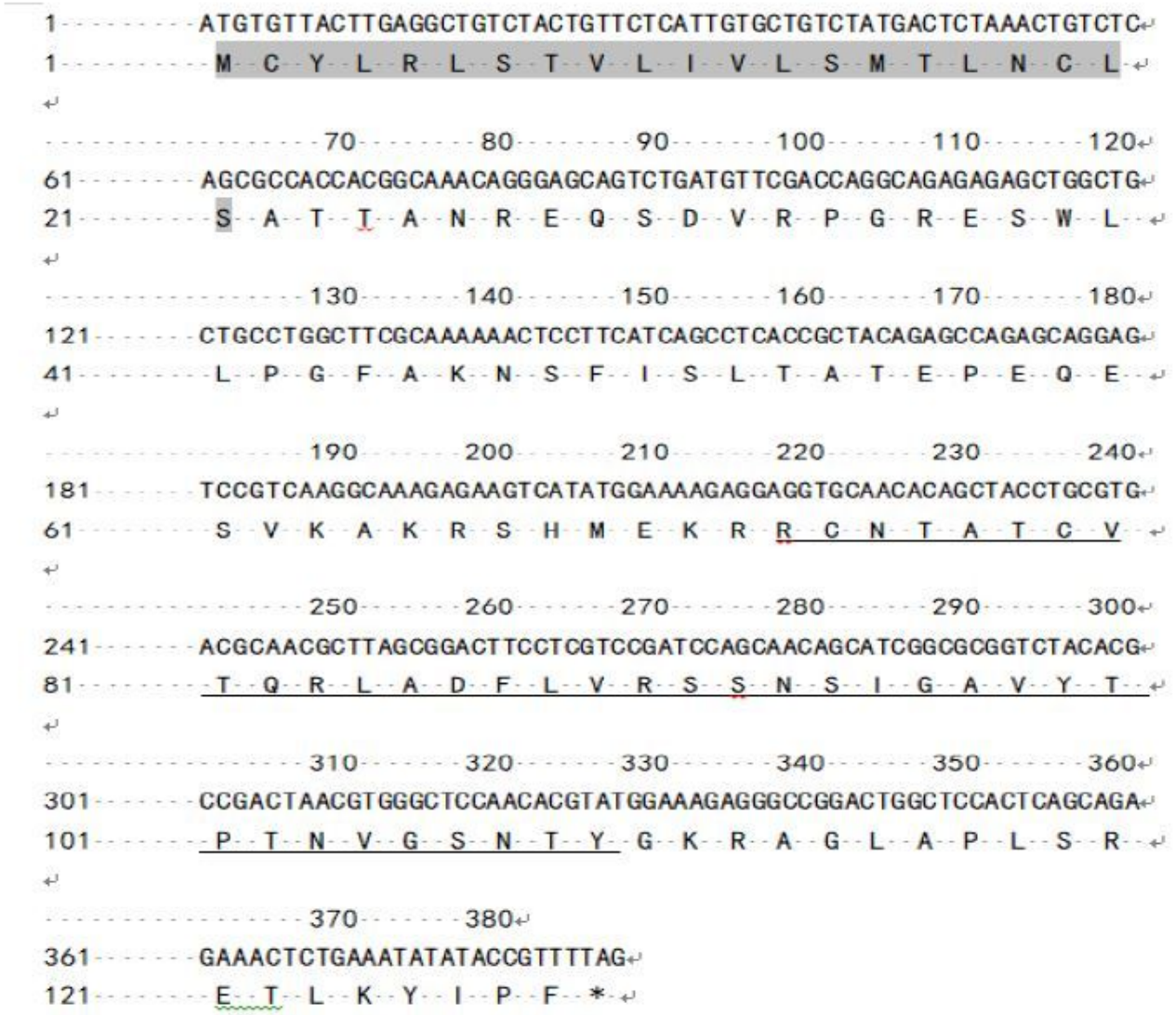
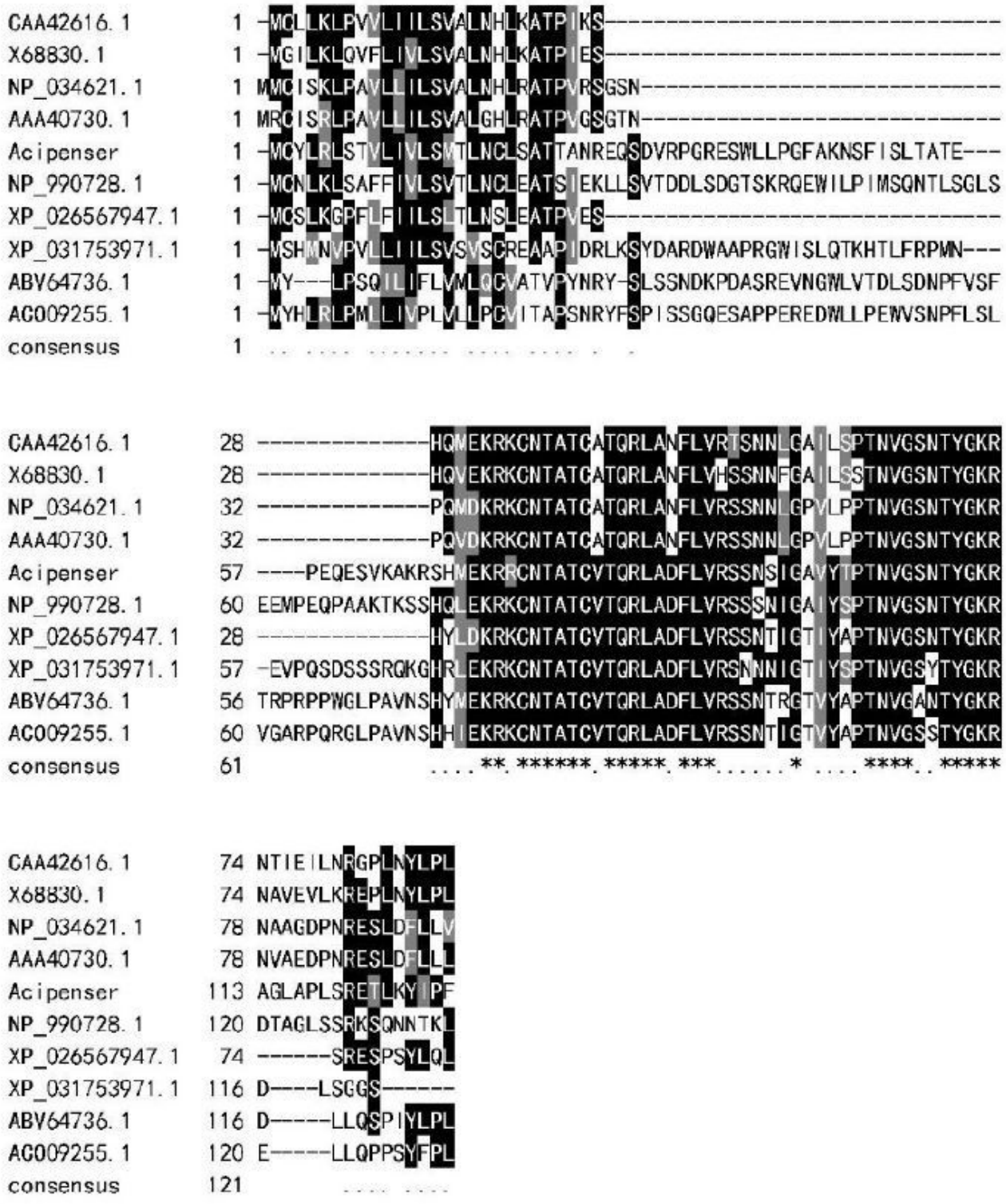



**Figure 1**

Amylin nucleotide sequence and presumed amino acid sequence of Siberian sturgeon. The gray area is the signal peptide, and the underlined area is the functional peptide of amylin.




## Figure 2

Reweight comparison of amino acid sequences of Amylin from different species. Species name and GenBank accession number: CAA42616.1 Canis lupus familiaris；X68830.1 Homo sapiens；NP_034621.1 Mus musculus；NP_990728.1 precursor Gallus gallus；Acipenser Baerii；XP_ 026567947.1e Pseudonaja textilis；XP_ 031753971.1 Xenopus tropicalis；ABV64736.1 Carassius auratus ；ACO09255.1 Osmerus

mordax AAA40730.1 Rattus norvegicus. The different colors of amino acids indicate the differences in amylin amino acids between different species.

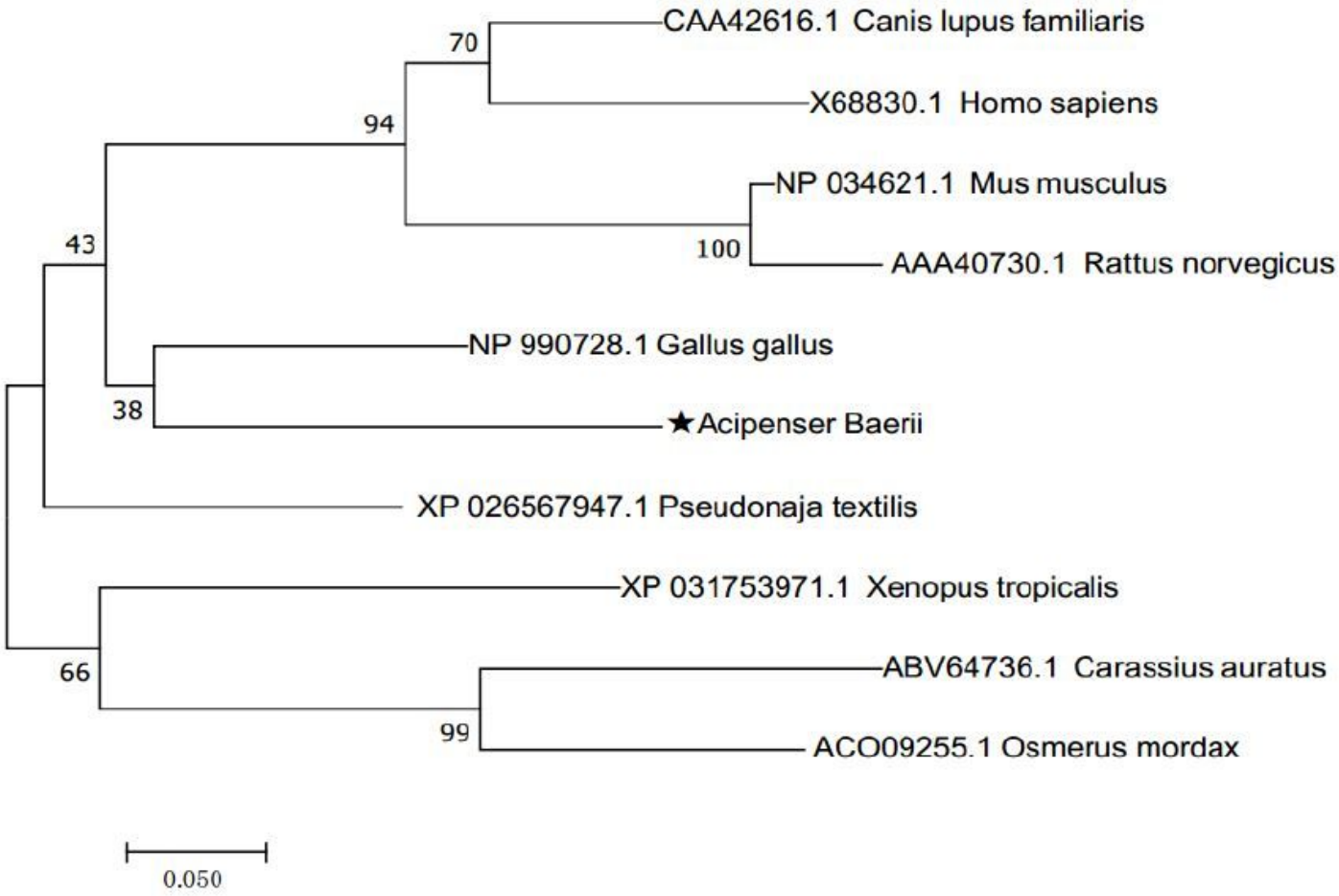



**Figure 3**

Evolutionary tree analysis of Amylin amino acids of different species. Species name and GenBank accession number: CAA42616.1 Canis lupus familiaris X68830.1 Homo sapiens NP_034621.1 Mus musculus NP_990728.1 precursor Gallus gallus Acipenser Baerii XP_026567947.1e Pseudonaja textilis XP_ 031753971.1 Xenopus tropicalis ABV64736.1 Carassius auratus ACO09255.1 Osmerus mordax AAA40730.1 Rattus norvegicus.

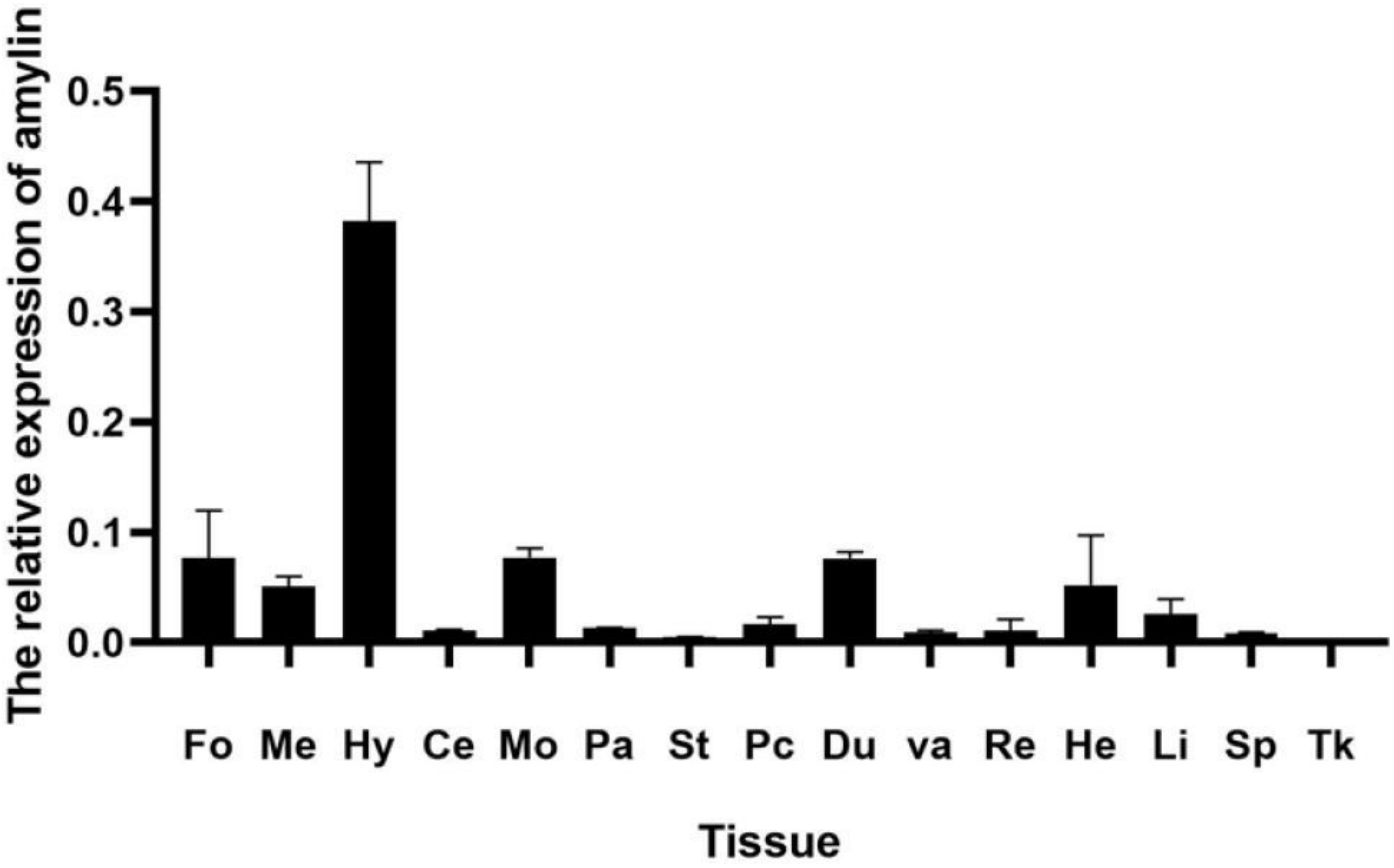



**Figure 4**

Tissue expression pattern of Amylin of Siberian sturgeon. Xsl-fo, forebrain; Me, the midbrain; HY, hypothalamus; Ce, the cerebellum; Mo, the medulla; Pa, pancreatic; St, the stomach; PC, pyloric caecum; Du, duodenum; Im va, valvular intestine; Re, rectum; He, the heart; Li, liver; Sp, spleen; Tk, kidney. Data are expressed as mean ±SEM (n = 6).

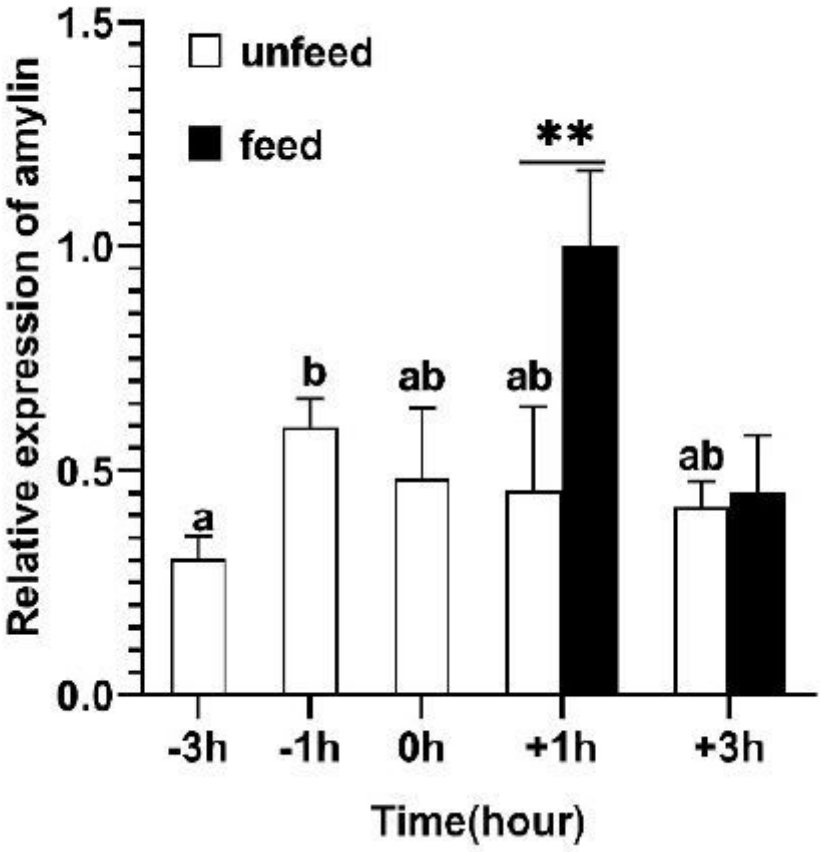



**Figure 5**

Expression of Amylin in the hypothalamus of Siberian sturgeon at periprandial. Data were expressed as mean ±SEM (n = 6) *, representing significant $P < 0.05$, and **, representing extremely significant $P < 0.01$.

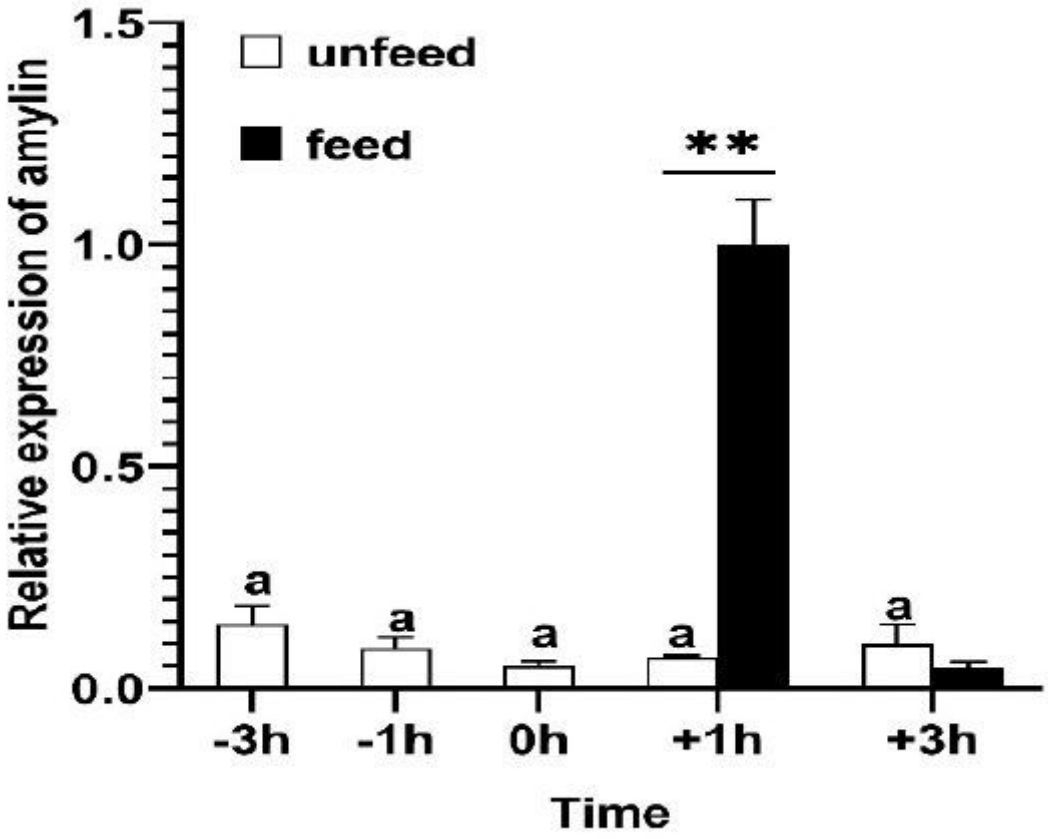



**Figure 6**

Amylin expression in duodenum of Siberian sturgeon at periprandial. Data were expressed as mean ±SEM (n = 6) *, representing significant $P < 0.05$, and **, representing extremely significant $P < 0.01$.

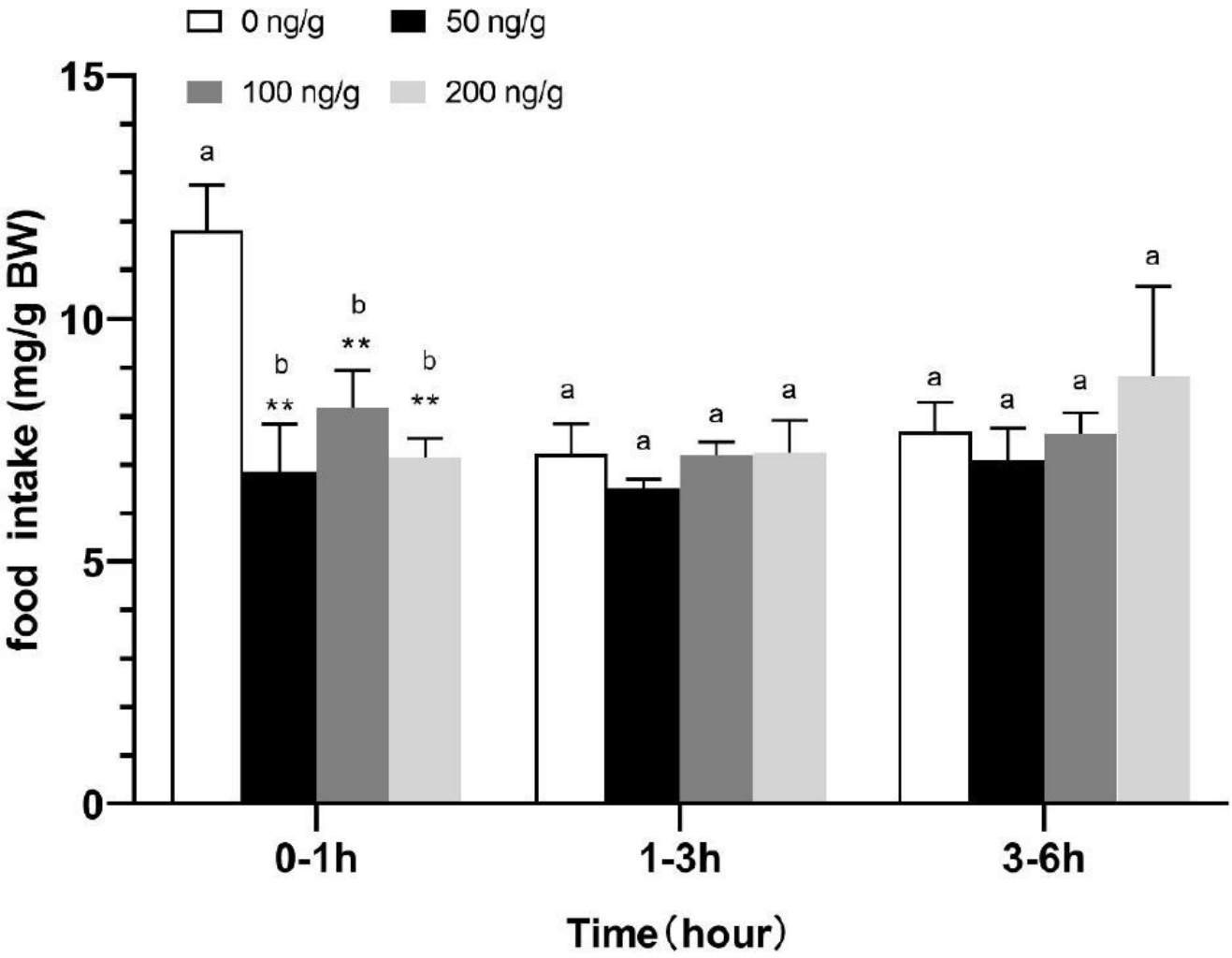



**Figure 7**

Effects of intraperitoneal injection of amylin on feed intake of Siberian sturgeon. Food intake was calculated at 1, 3, and 6h after injection. Data are expressed as mean ±SEM (n = 6). * represents significant difference $P < 0.05$, and ** represents extremely significant difference $P < 0.01$.

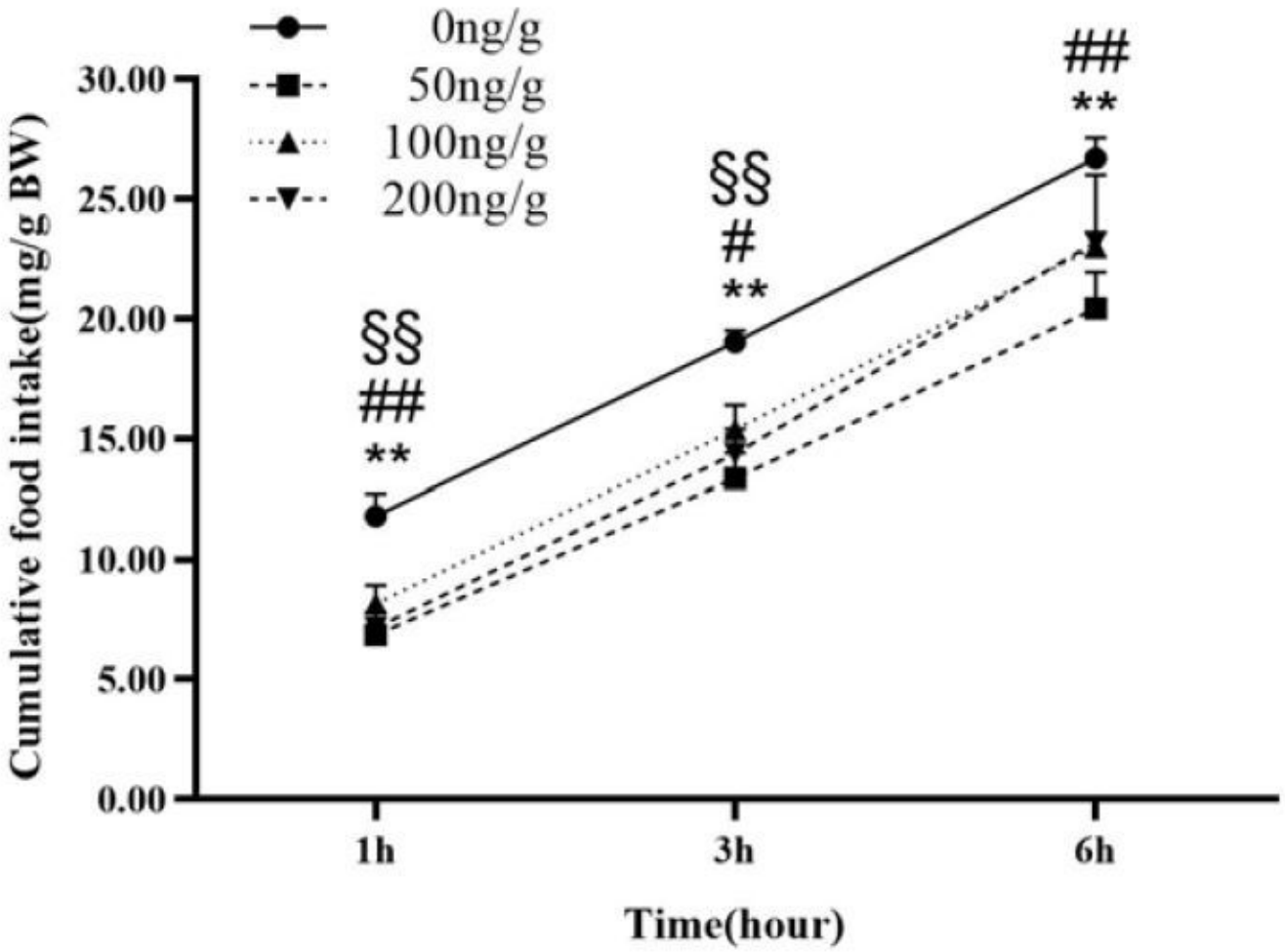



**Figure 8**

Effects of intraperitoneal injection of amylin on cumulative feed intake of Siberian sturgeon.* indicates significant difference between 50ng/g BW and normal saline control group;# indicates significant difference between 100ng/g BW and normal saline control group;§ indicates that there is a significant difference between 200ng/g BW and normal saline control group.

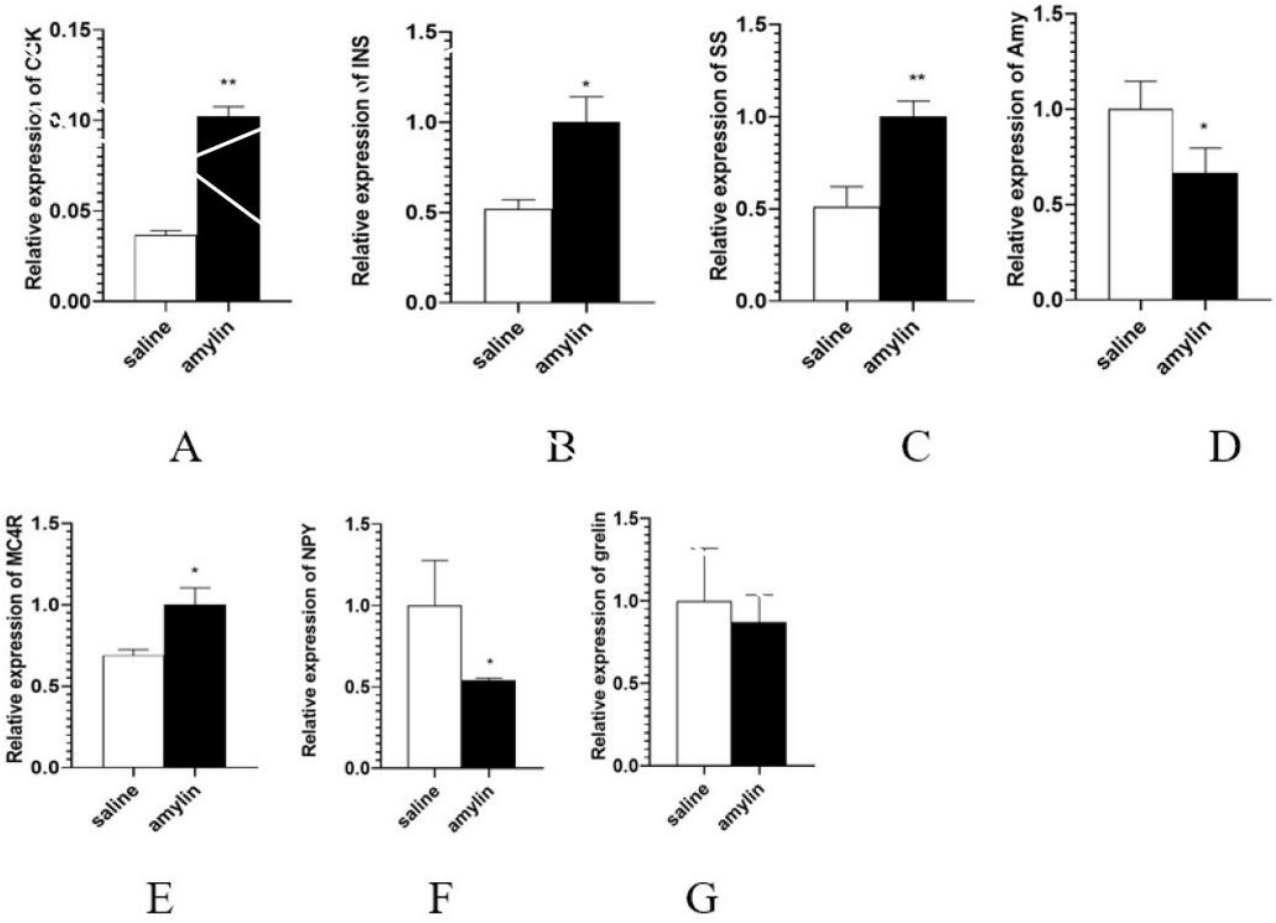



**Figure 9**

Effect of intraperitoneal injection of amylin on the expression of appetite factor of Siberian sturgeon 1 h later. The expression levels of CCK (A), insulin (B), somatostatin (C), amylin (D), MC4R (E), NPY (F) and ghrelin (G) were determined after 100 ng/g of amylin.Data are expressed as mean ±SEM (n = 6).* represents significant difference $P < 0.05$, and ** represents extremely significant difference $P < 0.01$.