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RELATIONSHIP BETWEEN FOOD INTAKE AND EXPRESSION OF
O-LINKED N-ACETYLGUCOSAMINE TRANSFERASE
MESSENGER RNA IN CHANNEL CATFISH

being

A Thesis Presented to the Graduate Faculty
of Fort Hays State University in
Partial Fulfilment of the Requirements for
the Degree of Master of Science

by

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B.S., Fort Hays State University

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This thesis for
the Master of Science Degree

by

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Chair, Supervisory Committee

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PREFACE

This thesis follows the guidelines of the Journal of Comparative Biochemistry and Physiology Part A-Comparative and Integrated Physiology.

ABSTRACT

Food intake regulation is a complex neural process that involves the coordination of multiple mechanisms. O-linked N-acetylglucosamine transferase (OGT) is a neural nutrient sensor that aids in regulating satiety in mammals. Compared to mammals, little is known about function and regulation of OGT expression in fish. It was hypothesized changes in food intake are associated with changes in OGT expression in channel catfish. The objectives of this study were to examine tissue distribution of OGT mRNA and determine the possible relationship between food intake and OGT mRNA in channel catfish. Screening of the catfish genome database yielded four highly homologous transcript variants. The predicted amino acid sequence of channel catfish OGT variants was highly homologous (>90%) to those of other fish and mammals. Expression of OGT was detected in many tissues including the heart, liver, spleen, kidney, and muscle, but was most readily detectable in the brain. Prolonged fasting, as well as fasting followed by refeeding, decreased expression of total OGT in the brain. In contrast, prolonged fasting increased expression of total OGT in the liver, and refeeding fish after fasting restored total OGT expression in the liver to a level similar to that of fish that received food daily. Additionally, a correlation between increased feeding and increased expression of total OGT was observed in the brain of channel catfish. Compared to total OGT, expression of OGT variant X1 and X3 was not affected by changes in food intake. These results suggest that OGT expression appears to be influenced by the nutritional status of channel catfish. The results of this study also indicate that changes in OGT expression are not associated with the expression of OGT variant X1 and X3.

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1. Introduction

Regulation of food intake is a complex neural process that involves behavioral components, hypothalamic control, endocrine function, and the interpretation of peripheral signals (Simpson et al., 2009; De and Dieguez, 2014). Food intake is regulated by the hypothalamus and brainstem in the central nervous system (CNS) and is controlled using orexigenic and anorectic neuropeptides in the peripheral nervous system (Lenard and Berthoud, 2008; Soria-Gómez et al., 2014). The hypothalamus interprets orexigenic and anorectic neuropeptide signals to stimulate or inhibit food intake, respectively (Lenard and Berthoud, 2008). Interaction of these neurotransmitters in the hypothalamus regulates appetite and satiety (Ahima and Antwi, 2008; Soria-Gómez et al., 2014). Feedback loops between the CNS and peripheral tissues play a critical role in the regulation of food intake by sending signals to the hypothalamus, which are used to determine the overall energy expenditure and energy intake of the body (Weiss, 2008). However, the brainstem regulates food intake by integrating short-term signals from the gastrointestinal tract that contribute mainly to meal termination (Soria-Gómez et al., 2014). Additionally, both food-seeking behavior and satiety perception play a role in meal initiation and termination, respectively, and are regulated by CNS mechanisms, including energy homeostasis, gastrointestinal hormones, and adiposity negative feedback (Morton et al., 2006).

O-linked N-acetylglucosamine transferase (OGT) is an evolutionarily conserved enzyme that catalyzes the O-linked glycosylation of proteins (Hart et al., 2011; Vella et al., 2013). In mice, OGT is located on the X chromosome, and the activity of its gene is

associated with various functions, including embryologic stem cell viability and nutrient sensing (Shafi et al., 2000; Vella et al., 2013; Lagerlöf et al., 2016). OGT is a neural nutrient sensor that aids in the regulation of food intake (Schwartz, 2016). In mice, the expression of OGT is highest in the hypothalamus, particularly in the paraventricular nucleus (PVN), but is also expressed in lower concentrations in peripheral tissues, such as the liver (Ruan et al., 2014; Schwartz, 2016). The PVN contains a high concentration of anorectic neurons that regulate satiety and energy expenditure (Schwartz and Woods, 2000; Morton et al., 2006). The expression of OGT controls satiation through the regulation of the thresholds associated with the satiety feedback loops (Lagerlöf et al., 2016). In OGT knockout mice, food intake increased drastically, resulting in massive weight gain (Schwartz, 2016). This significant weight gain is correlated to an increase in adiposity rather than increase in lean mass (Schwartz, 2016). OGT knockout mice also developed hyperphagia (Lagerlöf et al., 2016). During each meal, OGT knockout mice consumed more food and spent longer time consuming the meal rather than consuming food at a higher frequency (Lagerlöf et al., 2016). Additionally, OGT serves as a glucose sensor and expression of OGT in the liver regulates the gluconeogenesis pathway (Ruan et al., 2012; Bindsbøll et al., 2015; Pepe et al., 2017). Overexpression of OGT in the liver of mice suppresses the insulin response resulting in insulin resistance and dyslipidemia (Dias and Hart, 2007; Yang et al., 2008).

As in mammals, food intake in fish is regulated through complex mechanisms, including changes in the expression of mRNA encoding orexigenic and anorexic peptides (Volkoff, 2016). Changes in the expression of cocaine- and amphetamine-regulated

transcript (CART), neuropeptide Y (NPY), and pro-opiomelanocortin (POMC) in relation to changes in food intake have been investigated in several fish species, including zebrafish, salmon, and goldfish (Volkoff, 2016). However, many mechanisms that play a role in food intake currently remain unexplored.

Cocaine- and amphetamine-regulated transcript is a peptide that acts as an anorexic factor that inhibits appetite in mammals and fish (Volkoff, 2016). Expression of CART inhibits NPY function and fasting decreases the expression of CART in the brain of mammals and fish (Kobayashi et al., 2008; Zhang et al., 2018). In channel catfish (*Ictalurus punctatus*), there is a negative correlation between food intake and the expression of CART mRNA (Kobayashi et al., 2008). Therefore, a decrease in CART expression is associated with an increase in food intake, resulting in increased growth in channel catfish. This increased growth may be attributed to reduced inhibition of NPY in channel catfish.

Neuropeptide Y is highly abundant in the brain of mammals (Loh et al., 2015). However, in fish, NPY has a widespread distribution with the highest abundance in the brain and intestinal tract (Volkoff, 2016). NPY plays a role in the regulation of food intake by interacting with the hypothalamus and acting as an orexigenic factor (Silverstein and Plisetskaya, 2000; Volkoff, 2016). Yokobori et al. (2012) reported that fasting increased expression of NPY in the hypothalamus of zebrafish. Additionally, there is a positive correlation between food intake and the expression of NPY in channel catfish (Silverstein and Plisetskaya, 2000).

Pro-opiomelanocortin is shown to suppress appetite in mammals and fish and is primarily expressed in the pituitary gland and the hypothalamus (Ellacott and Cone, 2006; Volkoff, 2016). In most fish, POMC has been shown to inhibit food intake by inhibiting the NPY system and releasing α -melanocyte stimulating hormone (α -MSH) (McMinn et al., 2000; Volkoff, 2016; Steyn et al., 2017). In mice, a specific deletion of α -MSH receptors in the hypothalamus exhibit an obese phenotype, indicating that the expression of POMC is involved in food intake (Ellacott and Cone, 2006). In channel catfish, the correlation between POMC and food intake has not been previously studied.

In channel catfish, faster growth is correlated with an increase in food consumption (Kobayashi et al., 2008; Peterson et al., 2008), and genetic selection toward increased growth often leads to accumulation of fat in the abdomen (Li and Lovell, 1992; Kobayashi et al., 2015). Few studies have been conducted on genetic mechanisms that contribute to food intake in channel catfish (Silverstein et al., 2001; Peterson et al., 2012; Schroeter et al., 2015), however, the exact mechanism(s) associated with increased food intake and adiposity in response to genetic selection toward increased growth are unknown in channel catfish.

Based on observations in mice, it is possible that changes in expression of OGT might contribute to changes in food intake and subsequent changes in growth in channel catfish. However, the link between food regulation and the expression of OGT in channel catfish has not been previously explored. Furthermore, little is known about the expression of OGT, including its tissue distribution, in channel catfish.

Therefore, the objectives of this study are to examine the tissue distribution of OGT mRNA and to determine the possible relationship between food intake and the amount of OGT mRNA expressed in the tissues of channel catfish.

2. Materials and Methods

2.1. Animal care and maintenance

All studies involving the use of live fish were conducted according to the protocols approved by the Fort Hays State University Institutional Animal Care and Use Committee (protocol number 18-0001). Juvenile channel catfish were obtained from a local fish hatchery (Milford Fish Hatchery, Kansas Department of Wildlife, Parks, and Tourism, Milford, KS, USA). Fish were cultured in a commercially available recirculating zebrafish culture system that was modified to culture larger, warm water fish (Aquatic Enterprises Inc., Seattle, WA, USA). The system was equipped with twelve 40-liter tanks with a recirculating system that maintained water flow to ensure that water turned over twice daily. Fifty percent of the water was replaced every 7 days with dechlorinated tap water (pH=7.0, DO>9.0 mg/L) to maintain the quality of water within the culture system. Fish were maintained by feeding commercially available fish food (36% crude protein, Cargill Animal Feed, Minneapolis, MN, USA) once daily to visual satiety unless otherwise noted. Average water temperature was maintained at 24 °C. Fish were exposed to a natural daylight cycle with fluorescent light supplementation and were kept in a 14 light: 10 dark hour photoperiod throughout the study.

2.2. Identification of channel catfish OGT gene

The OGT transcripts were identified by searching channel catfish genome database available in GenBank (www.ncbi.nlm.nih.gov/genome/?term=CHANNEL+CATFISH). During screening of the database, four distinct transcripts that encoded 110 kDa subunits of OGT were

identified (GenBank Accession Number: XM_017473739, XM_017473740, XM_017473741, XM_017473742). These transcripts generated four distinct OGT variants (XP_017329228.1, XP_017329229.1, XP_017329230.1, XP_017329231.1). To identify the location of the OGT gene within channel catfish chromosomes, the OGT sequence was compared against channel catfish genome sequences available in the GenBank database using the blast RefSeq function (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The predicted amino acid sequence of the catfish OGT transcript variants was compared against sequences deposited in GenBank using the blastp function (<http://blast.ncbi.nlm.nih.gov/>). Each channel catfish OGT transcript variant was compared against two human (NP_858058.1 and NP_858059.1), two mouse (NP_631883.2 and NP_001277464.1), four zebrafish (NP_001017359.1, NP_001018115.1, NP_001018116.1, and NP_001018117.1), and eight Nile tilapia (XP_019223227.1, XP_019223229.1, XP_005467940.1, XP_003445936.1, XP_019223237.1, XP_013127170.1, XP_003445937.1, and XP_019223241.1) OGT transcript variants. Multiple alignments of OGT proteins, as well as a phylogenetic tree that demonstrated the evolutionary relationship among catfish OGT and OGT of other vertebrates, were generated using Geneious Software. Predicted domain structure of catfish OGT was determined using InterProScan sequence search software (<http://www.ebi.ac.uk/interpro/search/sequence-search>). Two separate real-time polymerase chain reaction (qRT-PCR) primers were designed based on the channel catfish OGT sequences. One primer set was designed to amplify all OGT transcripts (total OGT), whereas the other primer set was designed to amplify two specific OGT

transcript variants X1 and X3 (X1/X3 OGT). Both primer sets were designed so that one primer of each set will cover the exon-intron junction.

2.3. Tissue distribution of OGT mRNA

Tissue samples were collected from three sexually immature, juvenile catfish (22.8 ± 4.6 g) to examine distribution of OGT. Fish were euthanized by an overdose (0.3 g/L) of tricaine methane sulfonate (MS-222; Western Chemicals Inc, Ferndale, WA, USA). Approximately 100 mg of tissue were collected from spleen, trunk kidney, liver, heart, and muscle, as well as the whole brain from each fish. Tissue samples were placed in 1 ml of RNazol-RT (Molecular Research Center Inc., Cincinnati, OH, USA), flash frozen in liquid nitrogen, and stored at -80 °C until RNA isolation.

2.4. Relationship between food intake and OGT expression in channel catfish

2.4. 1: Effects of fasting on expression of OGT mRNA

Ninety-six juvenile channel catfish (19.1 ± 1.0 g) were used in this study. Fish were cultured in 12 tanks (n=8 fish per tank), and each tank was randomly assigned to one of three treatments: control, fasted, or refed (n=4 tanks per treatment). The study was 28 days in duration. The control group received food once daily to visual satiety throughout the experiment, whereas the fasted group did not receive food throughout the experiment. The fish assigned to the refed group were fasted for the first 14 days of the experiment, then received food daily for the subsequent 14 days. No mortalities occurred throughout the experiment.

All fish were anesthetized with 0.1 g/L of MS-222 and weighed on day 0, 14, and 28. On day 28, two randomly selected fish from each tank were euthanized with an overdose (0.3 g/L) of MS-222 and the brain, muscle, and liver were collected. Tissue samples were placed in 1 ml of RNazol-RT (Molecular Research Center Inc.), flash frozen in liquid nitrogen, and stored at -80 °C until RNA isolation.

2.4. 2: Effects of feeding frequency on expression of OGT mRNA

Ninety-six juvenile channel catfish (11.9 ± 1.0 g) were cultured in 12 tanks (7 to 9 fish per tank), and each tank was randomly assigned to one of three feeding treatments: control, overfed, or underfed (n=4 tanks per treatment). Fish assigned to the control group were fed once daily, whereas fish assigned to the overfed group were fed twice daily. Fish assigned to the underfed group were fed every other day. Food was offered at 0900 hours every day for 28 days for the control group. Fish assigned to the overfed group received food at 0900 and 1700 hours every day. Fish assigned to the underfed group received food at 0900 hours every 48 hours. All fish across treatment groups were fed to visual satiety. On day 21, two mortalities occurred in one control group tank but was caused by an issue unrelated to feeding treatment. The final number of fish in that tank at the end of the study was five.

All fish were anesthetized with 0.1 g/L of MS-222 and weighed on day 0, 14, and 28. On day 28, two fish from each tank were randomly selected and euthanized with an overdose (0.3 g/L) of MS-222 and the brain, liver, and muscle were collected. Tissue

samples were placed in 1 ml of RNAzol-RT (Molecular Research Center Inc.), flash frozen in liquid nitrogen, and stored at -80 °C until RNA isolation.

2.5. Total cellular RNA isolation and synthesis of complementary DNA (cDNA)

Total cellular RNA was extracted from each sample using a Direct-zol RNA MiniPrep kit (Zymo Research Corp., Irvine, CA, USA) according to manufacturer's instruction. Isolated RNA was treated with commercially available DNase I (TURBO DNA-free™; Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instruction to remove genomic DNA contamination. The quantity of DNase I treated RNA was measured by measuring UV absorbance at 260 nm using a Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific). Quality of RNA was estimated by calculating the ratio of UV absorbance at 260 and 280 nm. All RNA was stored at -80 °C until cDNA synthesis.

Using an iScript DNA synthesis kit (Bio-Rad Corporation, Hercules, CA, USA), the cDNA was synthesized from 1 µg of DNase I treated total cellular RNA according to the manufacturer's instructions. Quantity and quality of cDNA were measured by UV absorbance as described above. The cDNA was stored at -20 °C until analyses.

2.6. Real-time qRT-PCR

Expression of total OGT and X1/X3 OGT was measured by real-time polymerase chain reaction (qRT-PCR) using SYBR green technology. The primers used for OGT assays were designed based on the sequences identified during screening of the GenBank database described in Section 2.2 (Table 1). Two housekeeping genes, α -tubulin and β -microglobulin, were used as internal controls for examination of OGT tissue distribution,

whereas 18S rRNA was used as an internal control for food intake studies based on the results of previous studies (Small et al., 2008; Kobayashi et al., 2015). The sequence of primers used for respective internal control genes are shown in Table 1. For each gene that was measured, one primer was designed to overlap an exon-intron junction.

The reaction mixture for qRT-PCR consisted of 5 μ L SSoAdvanced SYBR Green Supermix (Bio-Rad), 2.5 pmol forward and reverse primers, and 2.5 μ L of DEPC-treated water (Thermo Fisher). Fifty ng of cDNA was added to each well. In non-template control wells (reactions without cDNA), 2 μ L of DEPC-water was added in place of cDNA. The final volume of reaction mixture was 10 μ L per well. Bio-Rad CFX96 real-time detection system (Bio-Rad) was used to perform qRT-PCR. Thermo cycle consisted of a hot start (95° for 30 s) followed by 42 cycles of 95 °C for 5 s and 60 °C for 5 s.

For the examination of tissue OGT distribution, the cycle threshold (CT) value of 2 internal control genes from each sample were averaged and subtracted from the CT value of OGT. Expression of total OGT and X1/X3 OGT in each sample was calculated by using $2^{-\Delta CT}$ method as described previously (Mamedova et al., 2010; Kobayashi et al., 2015). Tissue OGT expression was converted to relative expression by dividing expression of OGT ($2^{-\Delta CT}$) of each tissue sample with average $2^{-\Delta CT}$ value of OGT of the tissue with the lowest OGT expression ($2^{-\Delta CT}$). The $2^{-\Delta CT}$ value of muscle was used to calculate relative expression of both total OGT and X1/X3 OGT in various tissues. The tissue OGT expression was not statistically analyzed. In both food intake studies, CT value of 18S rRNA was subtracted from the CT value of OGT. Before statistical analysis, expression of respective OGT transcripts was converted to “fold change over

control” by dividing expression of OGT of each sample ($2^{-\Delta CT}$) with average $2^{-\Delta CT}$ value of OGT of the control group. In the fasting study, fish fed once daily served as control to convert expression of OGT in fasted and refed group. In feeding frequency study, fish fed once daily served as control to convert expression of OGT in overfed and underfed group.

2.7. Statistical analysis

Fish weight, measured in both food intake studies, was analyzed using the MIXED model procedure in SAS for repeated measure (SAS on Demand, SAS institute, Cary, NC, USA). The model included day, treatment, and interaction of day and treatment as fixed effects and tank identity within treatment as a random effect. Weight over day was modeled with covariance structures using first-order autoregressive because weight was measured at regular intervals. Data are presented as least square means \pm standard error.

Statistical analysis of OGT expression in both feeding studies was conducted with R (version 3.3.2) using a one-way analysis of variance (ANOVA) with feeding treatments as the independent variable and expression of OGT as the dependent variable. Statistically significant differences between treatments ($p < 0.05$) were confirmed using a Tukey’s test. Data are presented as means \pm standard deviation. In the fasting study, the overall effects of fasting, even when followed by refeeding, on total OGT expression in the brain were analyzed using a two-sample t-test with treatment as the independent variable and expression of OGT as the dependent variable. The data are presented as means \pm standard deviation. In addition, the relationship between frequency of feeding

and total OGT expression in the brain was analyzed using a linear regression with treatment as the independent variable and expression of OGT as the dependent variable.

When the p-value was less than 0.05 ($p < 0.05$) or less than 0.01 ($p < 0.01$), differences between means were considered statistically significant. When the p-value was less than 0.10 but greater than 0.05 ($p < 0.10$), differences between means were considered as tendency. Otherwise, differences between means were considered statistically not significant ($p > 0.10$).

3. Results

3.1. Identification of channel catfish OGT gene

Screening of GenBank yielded four highly homologous OGT sequences found in channel catfish. Figure 1 shows the amino acid sequence of OGT transcript X4 and indicates where the additional amino acid sequences are found in transcript variants X1, X2, and X3. Each transcript differed by the insert of a short amino acid sequence.

Table 2 shows the similarity of channel catfish OGT predicted amino acid sequences to the predicted OGT amino acid sequences of humans, mice, zebrafish, and Nile tilapia. Each channel catfish OGT transcript was compared to the OGT transcript variant with the highest amino acid similarity of the respective organism. The predicted amino acid sequence of channel catfish OGT transcript variants was highly similar to that of humans (91-93%), mice (91-93%), zebrafish (94-97%), and Nile tilapia (93-96%).

The multiple sequence alignment and phylogenetic tree generated using Geneious software are shown in Figure 2 and Figure 3, respectively. Analysis with InterProScan software showed that the predicted domain structure of catfish OGT contained multiple tetratricopeptide repeats at the N-terminal domain, which play a role in protein-protein interactions and substrate recognition (Figure 2). The C-terminal domain contains the OGT catalytic domain responsible for enzymatic activity of the gene (Figure 2). These domains are conserved in OGT of other fish and mammals (Figure 2). The comparison of OGT sequences against the channel catfish reference genome sequence database available in GenBank showed that all four OGT transcripts aligned with the sequence of channel catfish chromosome 8 (GenBank Accession Number: NC_030423.1).

3.2. Tissue distribution of OGT mRNA

The tissue distribution of OGT in three juvenile channel catfish was examined using qRT-PCR. Figure 4 shows the relative expression of total OGT in the tissues of channel catfish. Figure 5 shows the relative expression of X1/X3 OGT in the tissues of channel catfish. The expression of total OGT and X1/X3 OGT was detected in all sampled tissues. Both total OGT and X1/X3 OGT was more readily detectable in the brain compared to other tissues. Additionally, OGT was readily detectable in the liver tissue of channel catfish.

3.3. Relationship between food intake and OGT expression in channel catfish

3.3. 1: Effects of fasting on expression of OGT mRNA

Changes in body weight of fish during the experiment are shown in Figure 6. On day 0, the average weight of fish among the three groups was similar ($p > 0.10$). On days 14 and 28, the weight of fish in the fasted treatment was significantly less than that of control fish (17.6 ± 1.0 g on day 14, $p < 0.05$; 18.0 ± 1.0 g on day 28, $p < 0.01$). Similarly, on day 14, fish in the refed group weighed less than control fish on day 14 (16.4 ± 1.0 g, $p < 0.01$). Although fish assigned to refed group gained weight by day 28 (20.1 ± 1.0 g) compared to day 14, weight of refed group was significantly lower than that of the control group ($p < 0.01$).

Figure 7 shows total OGT expression in the brain of channel catfish on day 28. Initial analyses indicated that there was a tendency ($p < 0.10$) for OGT expression to differ among the three groups. To determine whether fasting at any time length affected

expression of OGT, the fasted and refed groups were combined and compared to the control group using a two-sample t-test (Figure 8). The expression of OGT in the combined fasted and refed groups was significantly lower ($p < 0.05$) than the expression of OGT in the control group. Figure 9 shows the X1/X3 OGT expression in the brain of channel catfish on day 28. In the brain, the expression of X1/X3 OGT was not significantly different ($p > 0.10$) between the fasted, refed, and control treatment.

Figure 10 shows the total OGT expression in the liver of channel catfish on day 28. The expression of total OGT was significantly ($p < 0.01$) greater in the fasted group compared to the control group. The expression of total OGT was similar between the refed and control group on day 28. Figure 11 shows the X1/X3 OGT expression in the liver of channel catfish on day 28. The X1/X3 OGT expression was not significantly different ($p > 0.10$) among the three treatments.

3.3. 2: Effects of feeding frequency on expression of OGT mRNA

Changes in body weight of fish during the experiment are shown in Figure 12. On day 0, the average fish weight of each treatment among the three groups was similar ($p > 0.10$). On day 14, fish in the overfed group were similar in weight to fish in the control group ($p > 0.10$). However, on day 28, the weight of fish in the overfed treatment was significantly greater than that of control fish (26.2 ± 1.0 g, $p < 0.01$). On days 14 and 28, the weight of fish in the underfed treatment was significantly lower than that of the control group (13.8 ± 1.0 g on day 14, $p < 0.05$; 16.0 ± 1.0 g, $p < 0.05$).

Figure 13 shows the total OGT expression in the brain on day 28. In the brain, the expression of total OGT was not significantly different ($p>0.10$) between the control, underfed, and overfed treatments. The linear regression analysis indicated that expression of total OGT had a tendency ($p<0.10$) to be correlated with increased feeding frequency in channel catfish. Figure 14 shows the X1/X3 OGT expression in the brain on day 28. The expression of X1/X3 OGT was not significantly different ($p>0.10$) among the three treatments.

Figure 15 shows the total OGT expression in the liver on day 28. The expression of total OGT was not significantly different ($p>0.10$) among the three treatments. Figure 16 shows the X1/X3 OGT expression in the liver on day 28. The expression of X1/X3 OGT was not significantly different ($p>0.10$) among the three treatments.

4. Discussion

Previous studies (Butkinaree et al., 2010; Ruba and Yang, 2016; Pepe et al., 2017) have shown that OGT glycosylates proteins involved in cellular processes, such as transcription and the stress response, and OGT modulates the function of these proteins by influencing the protein-protein interactions and protein localization. The evolution of the OGT gene appears to be highly conserved among vertebrates, and OGT transcripts are generated through alternative splicing (Hanover et al., 2003; Park et al., 2017). The results of this study showed that all four OGT transcripts aligned with the identical region of chromosome 8, suggesting that channel catfish OGT transcripts are generated from one gene via the process of alternative splicing as observed in mammals. In mammals, the OGT sequence has been localized on the X chromosome (Dias and Hart, 2007); however, in channel catfish, the OGT sequence has been mapped to chromosome 8. Whether the channel catfish OGT gene is sex-linked as observed in mammals is unclear.

Although the location of the OGT sequence within the genome differs among species, the predicted OGT amino acid sequences of channel catfish are highly similar to the predicted amino acid sequences in fish and mammals. These results suggest that the OGT sequence has been highly conserved throughout the evolutionary process, which indicates the functional importance of the gene in vertebrate animals. In mammals, OGT has highly conserved multiple tetratricopeptide repeats in the N-terminal domain and the OGT catalytic domain in the C-terminal domain (Dias and Hart, 2007). The analysis of predicted channel catfish OGT indicates that catfish OGT also contains tetratricopeptide repeats in the N-terminal domain and C-terminal catalytic domain. Additionally, the

amino acid sequence of channel catfish OGT found in these regions were highly homologous to those of mammalian OGT. The results of this study indicated that OGT in vertebrates appears to be highly conserved in its genome structure, as well as amino acid sequence. Furthermore, domain analysis showed a high degree of conservation in the two domains found in OGT, suggesting that OGT is evolutionarily highly conserved through evolution among vertebrates. Given the high degree of genetic and structural conservation observed between channel catfish OGT and those of other species, it is possible that OGT in channel catfish may influence processes such as food intake regulation and stress response.

In mammals, the expression of OGT is tissue dependent (Butkinaree et al., 2010). In mice, the highest expression of OGT occurs in the brain, specifically the hypothalamus, whereas expression of OGT is lower in peripheral tissue, including the liver (Ruan et al., 2014; Schwartz, 2016). In humans, the tissue distribution of OGT is highest in the pancreas and placenta, with notable expression in the brain and heart (Lubas et al., 1997). However, as in mice, the expression of OGT is lower in other peripheral tissues (Lubas et al., 1997). Similar to mammals, OGT was most readily detectable in the brain of channel catfish, and OGT was detected in all tissues examined, including the heart, kidney, muscle, and spleen at various detectability levels. OGT was also readily detectable in the liver of channel catfish.

Expression of OGT is regulated by various cellular signals, such as nutrient availability and stress (Butkinaree et al., 2010). In the brain of mice, a decreased expression of OGT is associated with an increase in food intake (Lagerlöf et al., 2016).

The present study showed that during fasting, total OGT expression had a tendency to decrease in the brain of the fasted and refed groups when compared to the control. The expression of total OGT in brain was significantly lower in fish that were fasted, either continuously for 28 days or for 14 days before they received food. This suggests that OGT may share similar anorectic functions to those observed in rodents (Lagerlöf et al., 2016). OGT expression in the brain was associated with increased food intake in mice. The results of this study agreed with a previous study where prolonged fasting decreased expression of CART in the brain of channel catfish (Kobayashi et al., 2008). Contrary to the changes in expression of CART in response to fasting and refeeding (Kobayashi et al., 2008), refeeding after prolonged fasting failed to restore the expression of OGT to a level similar to that of the control. However, refeeding after fasting failed to restore body weight, which was similarly observed in CART expression in channel catfish (Kobayashi et al., 2008). It is possible that the mechanism(s) that regulate expression of these genes may be different from each other. Alternatively, decreased expression of OGT in the brain of channel catfish after fasting may indicate that fish may be in the state of negative nutrient balance. The mechanism(s) responsible for decreased expression of OGT in the brain requires further study.

In the hypothalamus of mice, increased expression of OGT is associated with satiety and meal termination (Lagerlöf et al., 2016). In the feeding frequency study, the linear regression indicated that increased expression of total OGT in brain had a tendency to be associated with increased feeding frequency. These findings correspond with increased OGT expression in the brain of mice resulting in decreased food intake and

meal termination. The results of the two feeding studies indicate that in the brain, expression of total OGT may be influenced by changes in food intake. Additionally, in both feeding studies, the expression of X1/X3 OGT was similar among the three treatments in the brain, suggesting that the changes in OGT expression in response to changes in food intake may be attributed to transcripts X2 and X4.

In the liver of mice, OGT serves as a glucose sensor, and insulin influences the activation of OGT by regulating the specificity of substrate binding (Bindesbøll et al., 2015; Pepe et al., 2017). In the fasting study, the expression of total OGT increased significantly in the fasted group as compared to the control group. Given that OGT regulates gluconeogenesis in liver *in vitro* and *in vivo* (Ruan et al., 2012), it is possible that catfish require additional glucose during fasting. To meet the demand for glucose, OGT may be stimulating hepatic gluconeogenesis. Therefore, expression of total OGT is increased in the liver during fasting. Refeeding fish for 14 days after 14 days of fasting restored hepatic expression of total OGT to a level similar to that of the control group. Kobayashi et al. (2008) demonstrated that, in channel catfish, prolonged fasting (30 days) followed by 15 days of refeeding increased the expression of CART mRNA to levels observed in fish fed continuously for 45 days. This may suggest that in liver, OGT expression is highly sensitive to nutrient levels compared to brain. Additionally, the expression of X1/X3 OGT in the liver was similar among the three treatments, suggesting that, as in the brain, the increased OGT expression might be attributed to OGT transcripts X2 and X4. In the feeding frequency study, the expression of total OGT and X1/X3 OGT was similar among the three treatments. It is possible that the catfish maintained glucose

levels due to the exposure to food. This result suggests the frequency of feeding may not influence the expression of OGT in the liver of channel catfish.

One of the proteins critical for nutrient homeostasis is AMP-activated protein kinase (AMPK) (Hardie, 2014, 2015). Increasing evidence suggests that OGT and AMPK regulates nutrient-sensitive intracellular processes cooperatively and mediate cellular growth and metabolism (Hardie et al., 2012; Bullen et al., 2014). In the present study, hepatic expression of OGT increased in response to prolonged fasting. In a previous study (Evans et al., 2016), fasting increased expression of AMPK subunit mRNAs in liver of channel catfish. The results of this study suggest that hepatic expression of these mRNAs that encode enzymes critical for normal cellular growth and metabolism is highly sensitive to nutrient status in channel catfish. In contrast, brain expression of AMPK subunit mRNA was unaffected by fasting (Evans et al., 2016). This may suggest the liver is the chief organ that monitors nutrient homeostasis in channel catfish. However, whether OGT and AMPK share similar regulatory mechanism(s) is unclear. Furthermore, the exact mechanism(s) associated with regulation of hepatic OGT expression is unknown and requires further investigation.

In summary, this study was the first to characterize expression of OGT in channel catfish, and the first to explore the relationship between changes in food intake and expression of OGT. Predicted amino acid sequences of four channel catfish OGT transcript variants shared a high degree of sequence similarity with OGT variants of other fish species, as well as those of some mammals. As in mammals, the expression of OGT was more readily detectable in the brain of channel catfish compared to other tissues.

However, the expression of OGT was also readily detectable in the liver of channel catfish. Although four different OGT variants were found in channel catfish, changes in OGT expression in response to food intake appeared to be specific to variants X2 and X4, given the expression of OGT variants X1 and X3 did not appear to change in response to food intake. This study showed the expression of OGT in the brain was sensitive to changes in food intake, especially when fish were deprived of nutrients for a prolonged period. Contrary to the results from other studies, refeeding failed to restore the expression of OGT, suggesting that OGT mRNA expression may be regulated differently from the expression of other neurotransmitter mRNAs involved in food regulation. The expression of OGT in the liver was elevated when fish did not receive food for prolonged periods. However, refeeding restored expression of OGT to a level similar to that of fish that received food daily. This may suggest the expression of OGT is regulated differently between the brain and the liver. Alternatively, liver may be more sensitive to changes in nutrient level compared to brain. The exact mechanism(s) involved in regulation of OGT expressions in response to changes in food intake is unclear and needs to be investigated further.

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Table 1: List of primers used for qRT-PCR.

Primer	GenBank Accession #	Sequence
OGT X1/X3 sense	This manuscript	GTGTCTGCTCTTCAGTACAACC
OGT X1/X3 antisense	This manuscript	TAGCAAGCCTTTGCCTCTTC
Total OGT sense	This manuscript	CGATACACAAGGACTCTGGAAATA
Total OGT antisense	This manuscript	GCAGTAAGCATCTGGGAAGT
α -Tublin sense	CB938582	ATCCGTAAACTGGCTGACCA
α -Tublin sense	CB938582	CAATTAGGAGGGAAGTGAAG
β -Macroglobulin sense	AF016042	AAGGGATGGAAGTTTCATCTGACC
β -Macroglobulin antisense	AF016042	GGAATGAAGCCCAGGAGGTTTAC
18S rRNA sense	AF021880	CGGAGAGGGAGCCTGAGAA
18S rRNA Antisense	AF021880	CGTGTCGGGATTGGGTAATTTG

Table 2: Predicted channel catfish OGT amino acid sequences compared to OGT amino acid sequences of zebrafish (*Danio rerio*), Nile tilapia (*Oreochromis niloticus*), mice (*Mus musculus*), and humans (*Homo sapiens*) available in the GenBank database by using Blast software.

Channel Catfish OGT Variant	Species	GenBank Accession # (Transcript Variant)	Amino Acid Sequence
X1	<i>Danio rerio</i>	NP_001017359.1 (X1)	97%
	<i>Oreochromis niloticus</i>	XP_003445936.1 (X4)	95%
XP_017329228.1	<i>Mus musculus</i>	NP_631883.2 (X1)	92%
	<i>Homo sapiens</i>	NP_858058.1 (X1)	92%
X2	<i>Danio rerio</i>	NP_001018115.1 (X2)	97%
	<i>Oreochromis niloticus</i>	XP_003445937.1 (X7)	95%
XP_017329229.1	<i>Mus musculus</i>	NP_001277464.1 (X2)	92%
	<i>Homo sapiens</i>	NP_858059.1 (X2)	92%
X3	<i>Danio rerio</i>	NP_001018116.1 (X3)	97%
	<i>Oreochromis niloticus</i>	XP_003445936.1 (X4)	96%
XP_017329230.1	<i>Mus musculus</i>	NP_631883.2 (X1)	93%
	<i>Homo sapiens</i>	NP_858058.1 (X1)	93%
X4	<i>Danio rerio</i>	NP_001018117.1 (X4)	97%
	<i>Oreochromis niloticus</i>	XP_003445937.1 (X7)	96%
XP_017329231.1	<i>Mus musculus</i>	NP_001277464.1 (X2)	93%
	<i>Homo sapiens</i>	NP_858059.1 (X2)	93%

Figure 1: Predicted amino acid sequence of channel catfish OGT transcript variant X4 acquired using GenBank. The amino acids in red (with underline) indicate the additional amino acids found in OGT transcript variants X1 and X3. The amino acids in blue (with underline) indicate the additional amino acids found in transcript variants X1 and X2.

MASSVGNVADSTEPTKRMLSFQGLAELAHREYQSGDFEAAERHCMQLW
RQEPDNTGVLLLLSSIHFCRRLDRSAHFSTLAIKQNPMLAEAYSNLGNVF
KERGQLQEAIHYRHALRLKPDFIDGYINLAAALVAAGDMEGAVQAYVS
ALQYNPDLYCVRSDLGNLLKALGRLEEAKACYLKAIETQPNFAVAWSNL
GCVFNAQGEIWLAIHHFEKAVTLDPNFLDAYINLGNVLKEARIFDRAVAG
YLRALSLSPNHAVVHGNLACVYYEQGLIDLAIPTYRAIELQPHFPDAYCN
LANALKEKGNVSEAEECYNTALRLCPTHADSLNNLANIKREQGNIEEAVQ
LYRKALEVFPEFAAAHSNLA SVLQQQKLEALMHYKEAIRISPTFADAY
SNMGNTLKEMQDVQGALQCYTRAIQINPAFADAHSNLA SIHKDSGNIPEAI
ASYRTALKLKPDPDAYCNLAHCLQIVCDWTDYDERMKKLVSIVADQLE
KNRLPSVHPHHSMLYPLSHGFRKAIAERHGNLCLDKINALHKPAYEHPKD
LKASGGRLRVGYVSSDFGNHPTSHLMQSIPGMHNSEKFEVFCYALSPDDS
TNFRVKVIAEAHHFTDLSQITCNGKAADRIHQDGIHILVNMNGYTKGARN
ELFALRPAPIQAMWLGYPGTSGAPFMDYIITDKETSPVEVAEQYSEK MAY
MPNTFFIGDHANMFPHLKKKAVIDFKSNGHIFDNRIVLNGIDLKAFDLSLP
DVKVIKMECDSQESTDNNGALSMPVIPMNTAAEAIINMINQGQIQVTINSF
TVSNGLATTQIFTVEEVVSGTVALQINNKAATGEEVPRTIVVTTTRSQYG
LPEDSIVYCNFNQLYKIDPPTLQMWANILKRVPN SVLWLLRFPVAVGEPNIQ
QYAQSMGLAASRIIFSPVAPKEEHVRRGQLADVCLDTPLCNGHTTGMDVL
WAGTPMVTMPGETLASRVAASQLTCLGCPELIAQSRQEYEDVAVKLGTD
MDYLKIRSRVWKQRICSPFN TKQYTVDLERLYLQMWEEHHASGGKPDH
LVKMQLLDSSESA

Figure 2: Multiple sequence alignment comparing channel catfish OGT amino acid sequences to OGT amino acid sequences of selected mammals and fish.

	1	10	20	30	40	50	60	70	80	90	100	110	120	130	140
1. <i>Ictalurus punctatus</i> - X1	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
	N-Terminal														
2. <i>Danio rerio</i> - X1	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
3. <i>Danio rerio</i> - X2	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
4. <i>Danio rerio</i> - X3	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
5. <i>Danio rerio</i> - X4	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
6. <i>Homo sapiens</i> - X1	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
7. <i>Homo sapiens</i> - X2	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
8. <i>Ictalurus punctatus</i> - X2	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
9. <i>Ictalurus punctatus</i> - X3	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
10. <i>Ictalurus punctatus</i> - X4	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
11. <i>Mus musculus</i> - X1	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
12. <i>Mus musculus</i> - X2	MASSVGHVAD STEPTERMLK FQGLAELAHR ETQSGDPEAA ERMCMQLWRQ EPDHTGULLL LSSIMHFQCR LDRSAHTSTL AIKGNPMLAE AYSHLGHVFK ERGQLQEAIE HYHMLRLKP DFIDGYINLA AALVAAGDME														
	150	160	170	180	190	200	210	220	230	240	250	260	270	280	
1. <i>Ictalurus punctatus</i> - X1	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
	N-Terminal														
2. <i>Danio rerio</i> - X1	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
3. <i>Danio rerio</i> - X2	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
4. <i>Danio rerio</i> - X3	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
5. <i>Danio rerio</i> - X4	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
6. <i>Homo sapiens</i> - X1	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
7. <i>Homo sapiens</i> - X2	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
8. <i>Ictalurus punctatus</i> - X2	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
9. <i>Ictalurus punctatus</i> - X3	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
10. <i>Ictalurus punctatus</i> - X4	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
11. <i>Mus musculus</i> - X1	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
12. <i>Mus musculus</i> - X2	GAUQAVYSAL QYNPDLVCVR SDLGHLKAL GRLEEAKACY LKAIEIQPHF AVAWSHLGCV FNAQGEIWLAE IMHFKAUATL DPNFLDAYIN LGVULEKARI FDRAVAGYLR ALSLSPPHVAU VHGHLACVYV EQGLIDLIAID														
	290	300	310	320	330	340	350	360	370	380	390	400	410	420	
1. <i>Ictalurus punctatus</i> - X1	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
	N-Terminal														
2. <i>Danio rerio</i> - X1	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
3. <i>Danio rerio</i> - X2	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
4. <i>Danio rerio</i> - X3	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
5. <i>Danio rerio</i> - X4	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
6. <i>Homo sapiens</i> - X1	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
7. <i>Homo sapiens</i> - X2	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
8. <i>Ictalurus punctatus</i> - X2	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
9. <i>Ictalurus punctatus</i> - X3	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
10. <i>Ictalurus punctatus</i> - X4	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
11. <i>Mus musculus</i> - X1	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
12. <i>Mus musculus</i> - X2	TYRRAIELQIP HFPDAYCHLA NALKERGVHS EAEECVHTAL RLCPHTADSL HNLAIIRKED GWIEEAUPLY RKALEVFFPEP AAAHSHLASV LQQQGLQEA LMHYKEAIRI SPTTADAYSH MGHITLKEMQD VQGLQCYTR														
	430	440	450	460	470	480	490	500	510	520	530	540	550	560	
1. <i>Ictalurus punctatus</i> - X1	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
	N-Terminal														
2. <i>Danio rerio</i> - X1	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
3. <i>Danio rerio</i> - X2	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
4. <i>Danio rerio</i> - X3	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
5. <i>Danio rerio</i> - X4	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
6. <i>Homo sapiens</i> - X1	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
7. <i>Homo sapiens</i> - X2	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
8. <i>Ictalurus punctatus</i> - X2	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
9. <i>Ictalurus punctatus</i> - X3	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
10. <i>Ictalurus punctatus</i> - X4	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
11. <i>Mus musculus</i> - X1	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														
12. <i>Mus musculus</i> - X2	AIQIHFAFAD AHSRLASINR DSGHIEPAIA SYRTALKLRF DFPDAYCHLA KCLQIVCDWT DYDERMHKLV SI VADQLLEK RLPSVHPHNS MLYPLSHGFR KAIAERHGHFL CLDKINALHK PAYERPKEDLK ASGGRLRUGY														

	570	580	590	600	610	620	630	640	650	660	670	680	690	700
1. Ictalurus punctatus - X1	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
	C-Terminal Domain													
2. Danio rerio - X1	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
3. Danio rerio - X2	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
4. Danio rerio - X3	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
5. Danio rerio - X4	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
6. Homo sapiens - X1	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
7. Homo sapiens - X2	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
8. Ictalurus punctatus - X2	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
9. Ictalurus punctatus - X3	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
10. Ictalurus punctatus - X4	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
11. Mus musculus - X1	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
12. Mus musculus - X2	VSSDFGHRPT SMLMQRIPGM HNSKEFEVFC YALSPPDDSTN FRUVVIAEAM HTTDLQSLITC HGKAADRIHQ DGIRILVUMNH GYTGARNEEL FALRPAPIQA MWLGYPTSG AFIMDYIITD KETSPEVAE QSEKMYMP													
	710	720	730	740	750	760	770	780	790	800	810	820	830	840
1. Ictalurus punctatus - X1	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
	C-Terminal Domain													
2. Danio rerio - X1	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
3. Danio rerio - X2	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
4. Danio rerio - X3	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
5. Danio rerio - X4	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
6. Homo sapiens - X1	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
7. Homo sapiens - X2	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
8. Ictalurus punctatus - X2	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
9. Ictalurus punctatus - X3	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
10. Ictalurus punctatus - X4	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
11. Mus musculus - X1	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
12. Mus musculus - X2	HTFFIGDHAM HFPNLEKKAVIDFKSNGHIF DRIIVLHGID LKAFLDLSDP UKVIMKCD - D SQESTD-HHG ALSMFPVIMH TAAEAIIHMI HGGQIQVTIN SFTVSHGLAT TQIFTVEEV VSGTVALQIN HKAATGEVFP													
	850	860	870	880	890	900	910	920	930	940	950	960	970	980
1. Ictalurus punctatus - X1	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
	C-Terminal Domain													
2. Danio rerio - X1	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
3. Danio rerio - X2	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
4. Danio rerio - X3	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
5. Danio rerio - X4	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
6. Homo sapiens - X1	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
7. Homo sapiens - X2	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
8. Ictalurus punctatus - X2	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
9. Ictalurus punctatus - X3	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
10. Ictalurus punctatus - X4	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
11. Mus musculus - X1	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
12. Mus musculus - X2	RTIVUTTRG YGLPEDIIVY CNFNQLYKID PPTLQMWANI LKRVVNSVLV LLEFFAUGEP HIQQYAQSMG LAASRIITSP VAPKEENVR GQLADVCLDT PLNGHTTGM DVLWAGTFMV TMPGETLASE VAASQLTCLG													
	990	1,000	1,010	1,020	1,030	1,040	1,050	1,060	1,064					
1. Ictalurus punctatus - X1	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
	C-Terminal Domain													
2. Danio rerio - X1	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
3. Danio rerio - X2	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
4. Danio rerio - X3	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
5. Danio rerio - X4	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
6. Homo sapiens - X1	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
7. Homo sapiens - X2	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
8. Ictalurus punctatus - X2	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
9. Ictalurus punctatus - X3	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
10. Ictalurus punctatus - X4	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
11. Mus musculus - X1	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													
12. Mus musculus - X2	CPFLIAQSRQ EYEDVAVELG TDMYDLEKIR SRVWVQRICS PLNTKQYTV DLERLYLQMV EHHASGKPPD HLVMGLLDS SESA													

Figure 3: Phylogenetic tree indicating the evolutionary relationship of channel catfish (*Ictalurus punctatus*), zebrafish (*Danio rerio*), mice (*Mus musculus*), and humans (*Homo sapiens*).

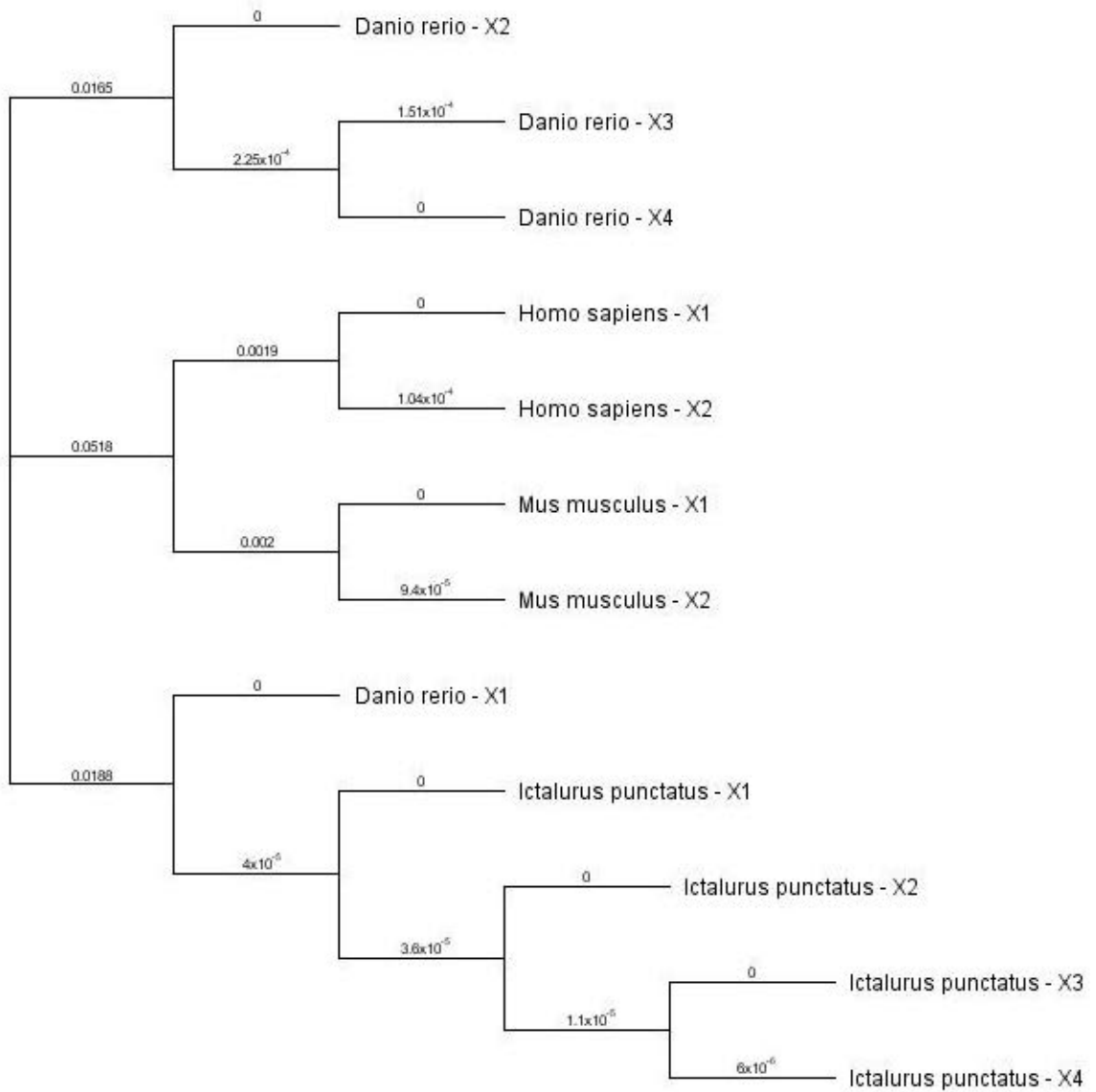


Figure 4: Tissue distribution of total OGT expression was measured using qRT-PCR. OGT expression in each tissue was corrected to relative expression over OGT expression in muscle (relative expression \pm SEM; n= 3 juvenile channel catfish).

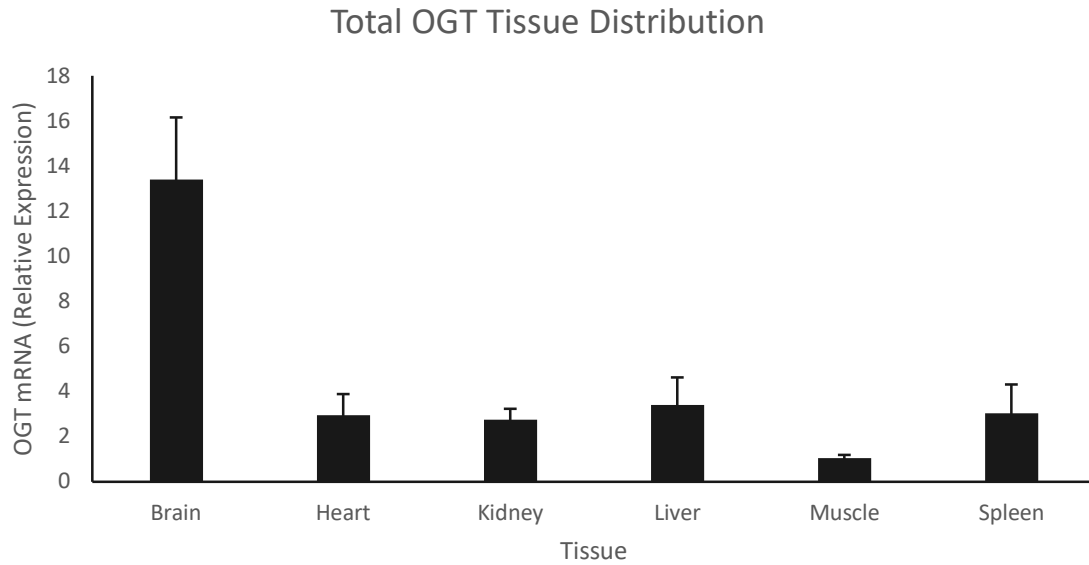


Figure 5: Tissue distribution of X1/X3 OGT expression was measured using qRT-PCR. OGT expression in each tissue was corrected to relative expression over OGT expression in muscle (relative expression \pm SEM; n=3 juvenile channel catfish).

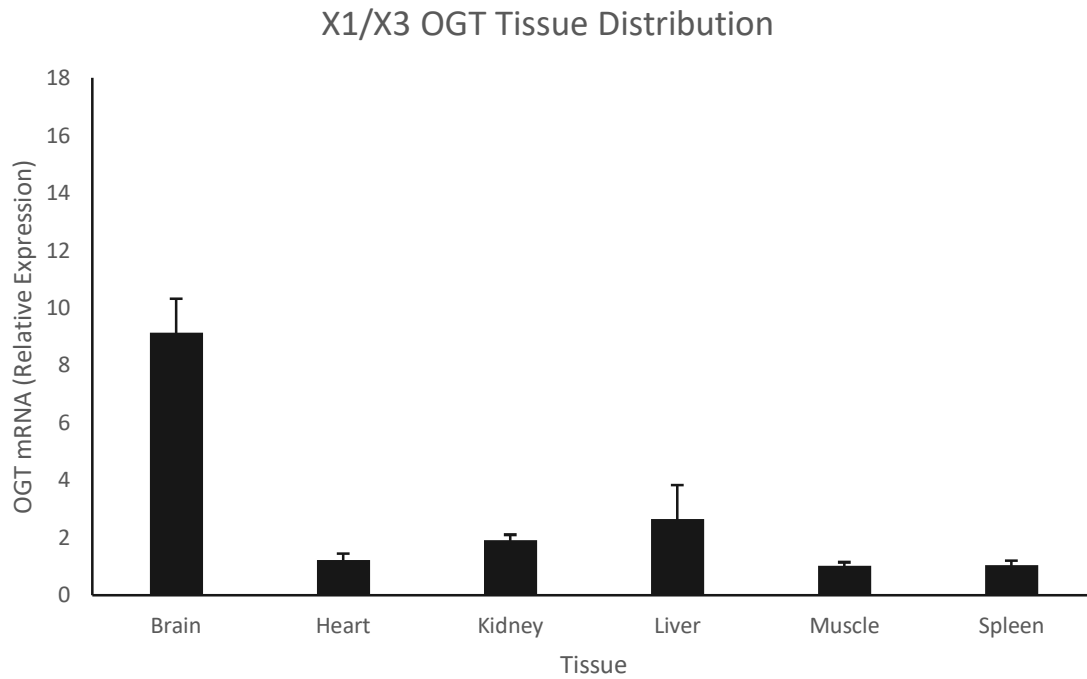


Figure 6: Changes in body weight (g) of juvenile channel catfish assigned to feeding treatments (LS Means \pm SEM; n= 8 fish per tank, 4 tanks per treatment; *p<0.05, **p<0.01). Treatments: fed daily (control), fasted (fasted), fasted for 14 days followed by 14 days of daily feeding (refed).

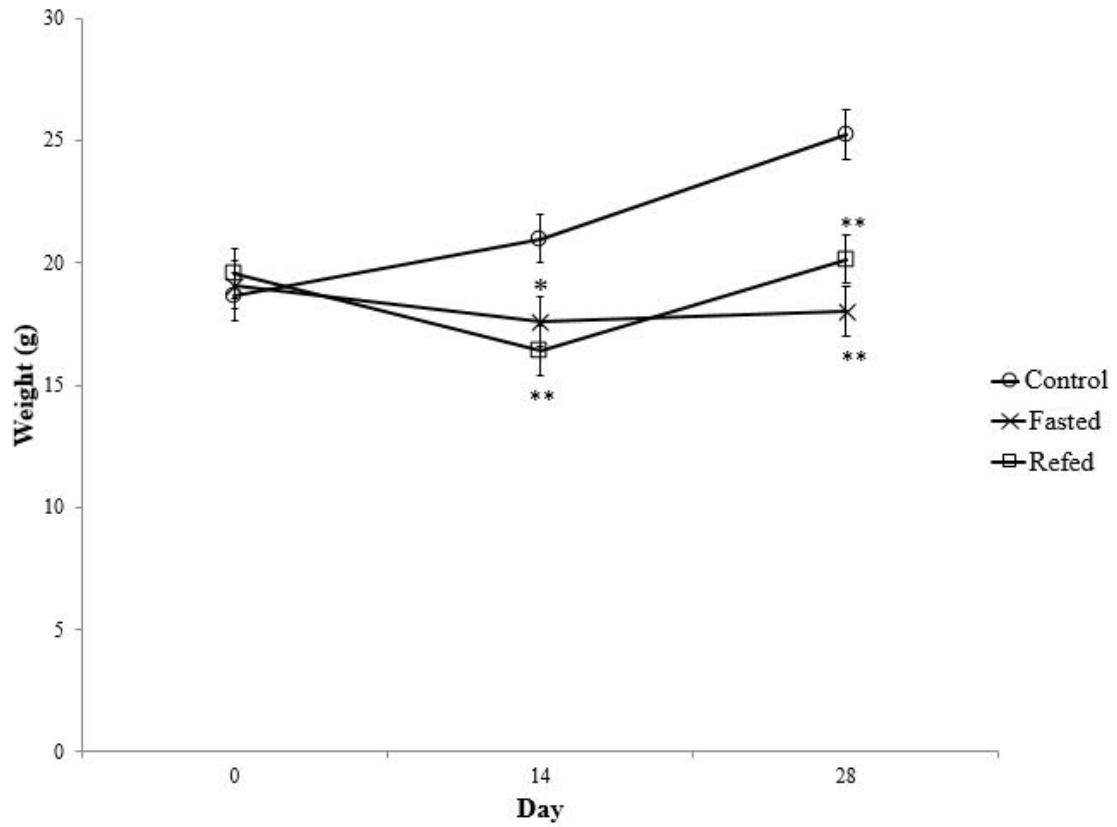


Figure 7: Relative expression of total OGT in brain of channel catfish after 28 day feeding treatment (relative expression \pm SD; n= 2 fish per tank, 4 tanks per treatment; p<0.10). Treatments: fed daily (control), fasted (fast), fasted for 14 days followed by 14 days of daily feeding (refed).

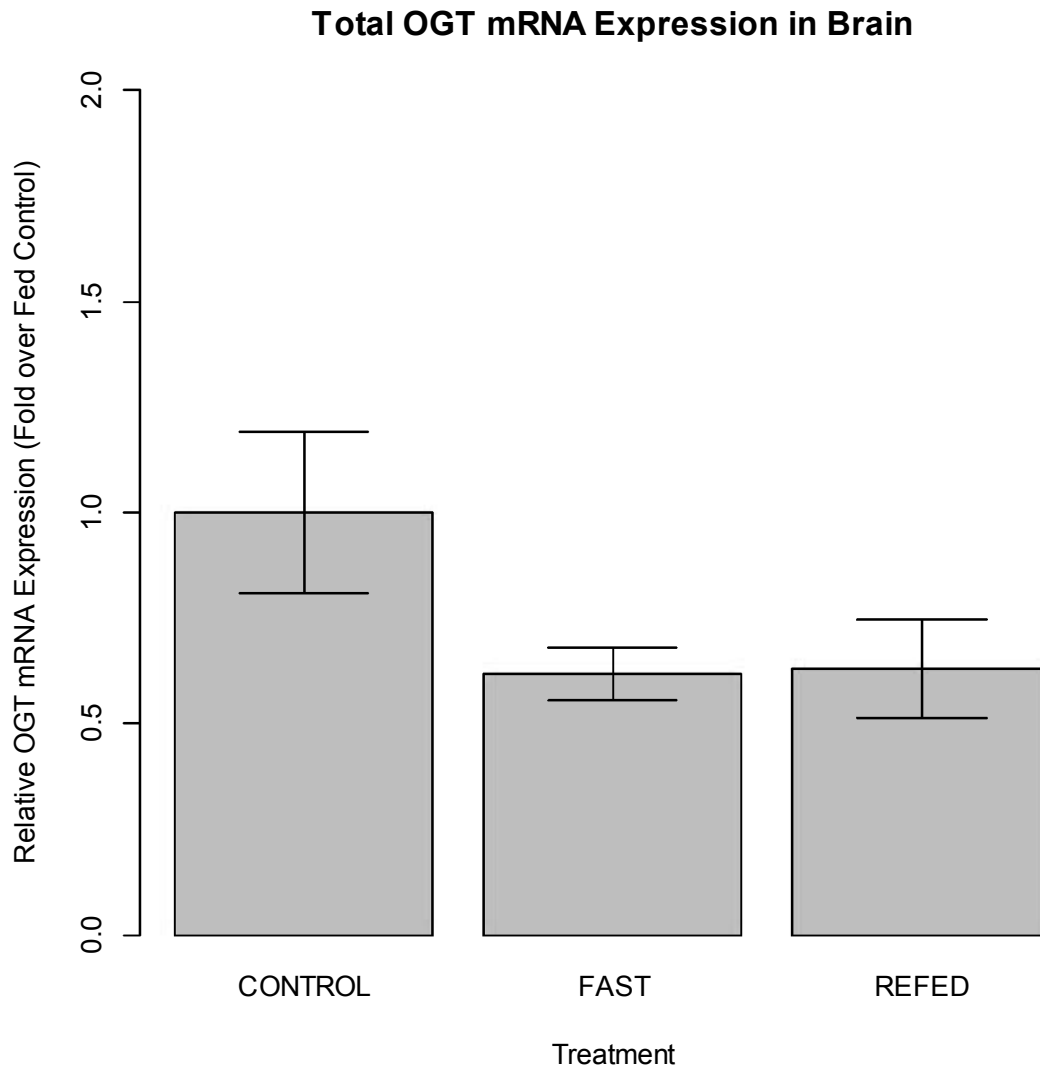


Figure 8: Relative expression of total OGT in the brain of channel catfish after 28 day feeding treatment (relative expression \pm SD; n=2 fish per tank, 4 tanks (control) or 8 tanks (fast/refed) per treatment, *p<0.05). Treatments: fed daily (control), fasted continuously or fasted for 14 days followed by 14 days of daily feeding (fast/refed).

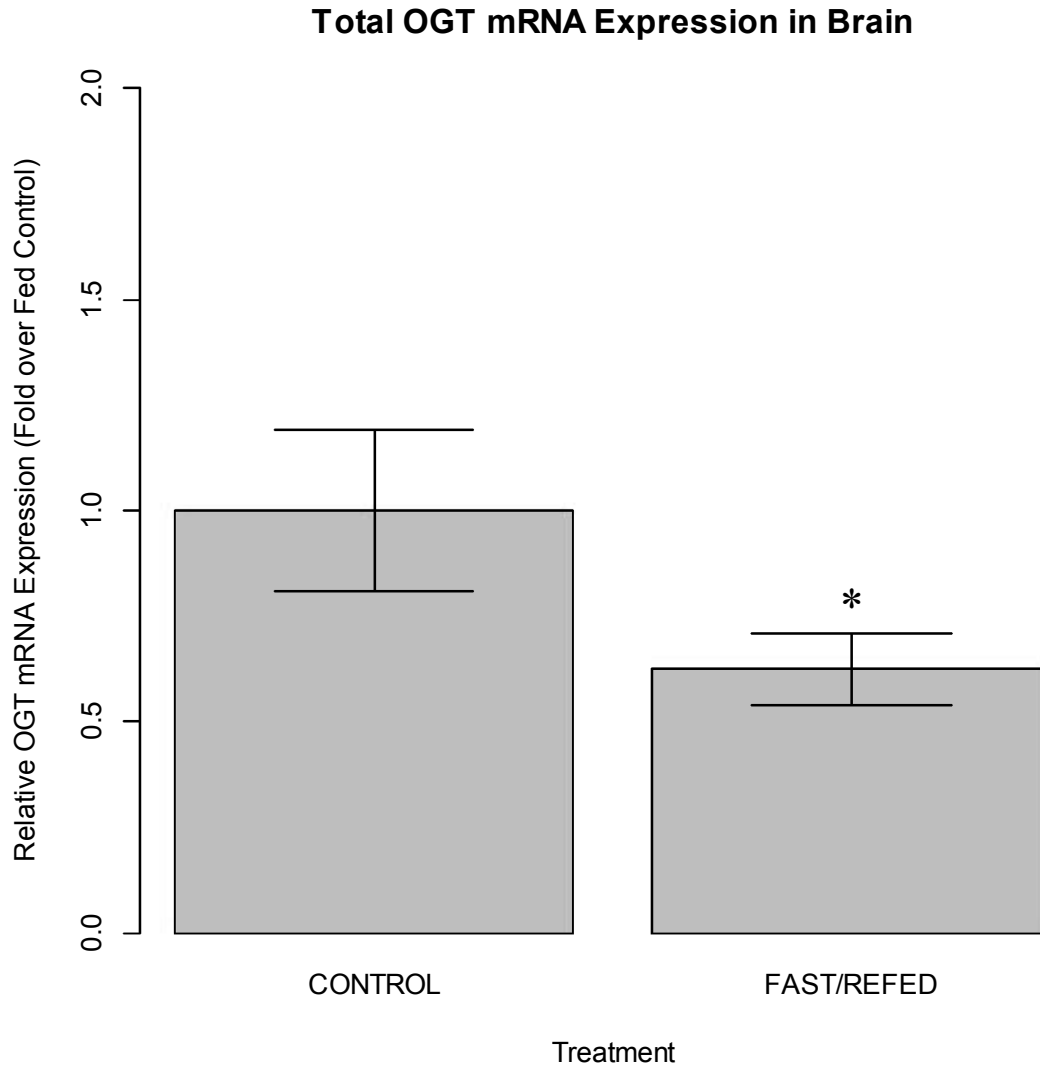


Figure 9: Relative expression of X1/X3 OGT in the brain of channel catfish after 28 day feeding treatment (relative expression \pm SD; n= 2 fish per tank, 4 tanks per treatment; $p>0.10$). Treatments: fed daily (control), fasted (fast), fasted for 14 days followed by 14 days of daily feeding (refed).

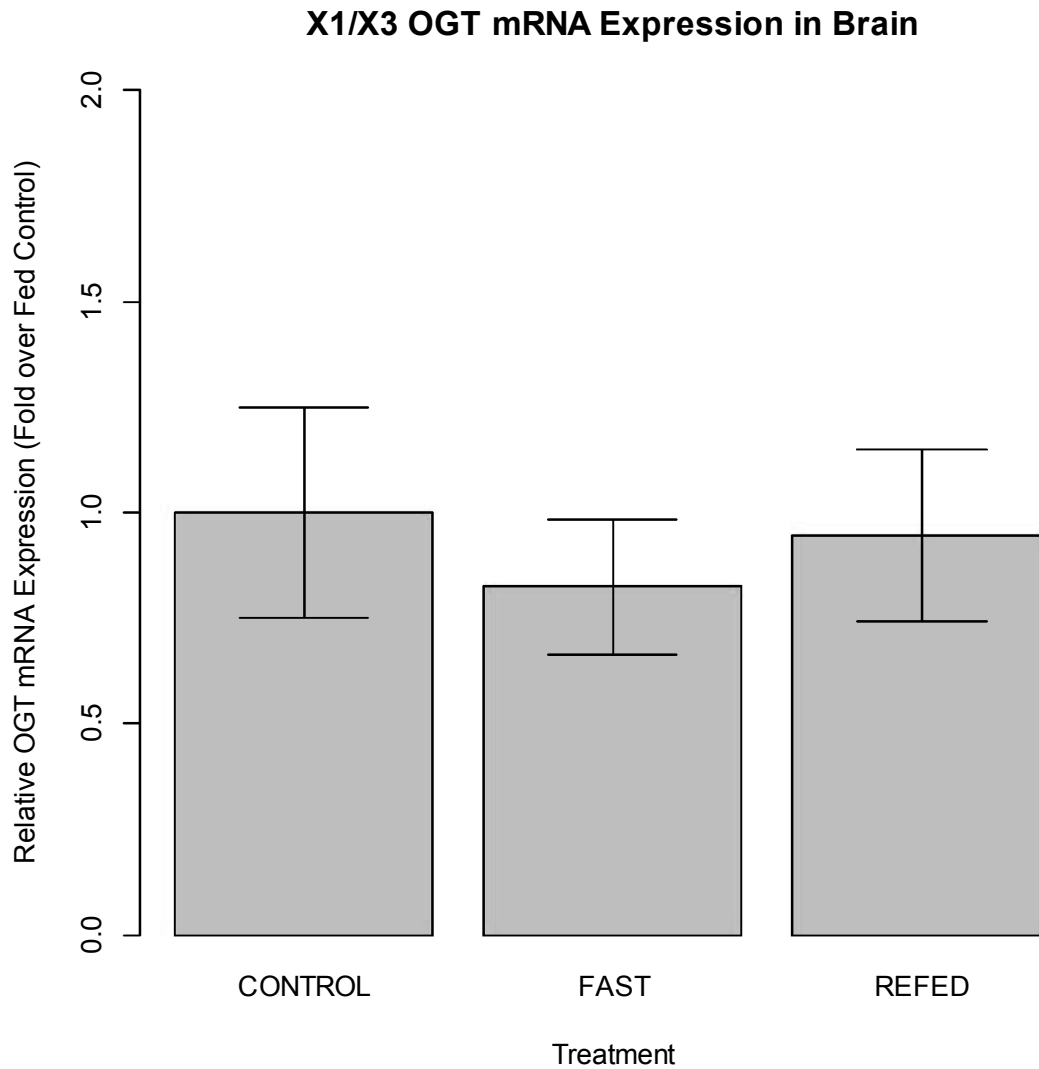


Figure 10: Relative expression of total OGT in the liver of channel catfish after 28 days of feeding treatment (relative expression \pm SD; n= 2 fish per tank, 4 tanks per treatment; *p<0.05). Treatments: fed daily (control), fasted (fast), fasted for 14 days followed by 14 days of daily feeding (refed).

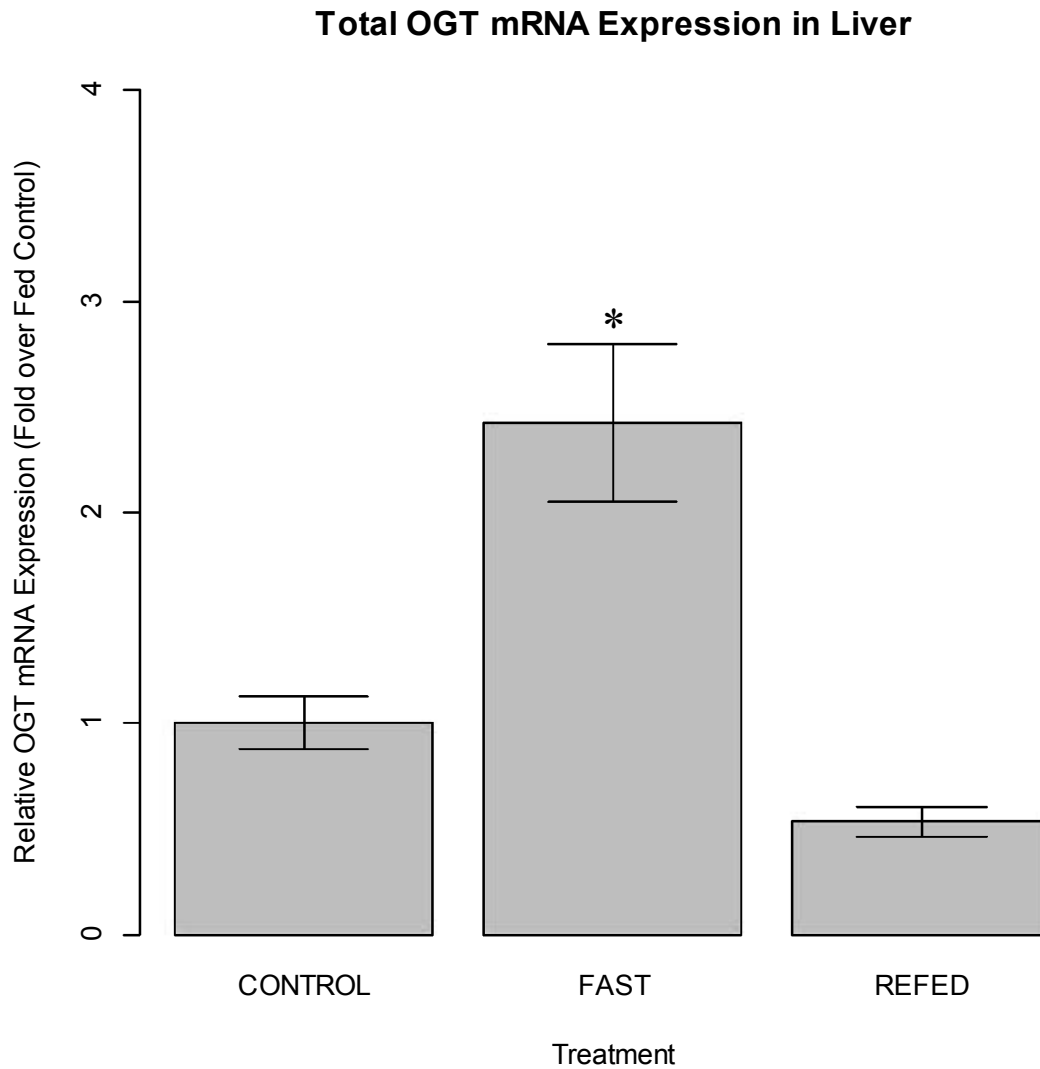


Figure 11: Relative expression of X1/X3 OGT in the liver of channel catfish after 28 day feeding treatment (relative expression \pm SD; n= 2 fish per tank, 4 tanks per treatment; p>0.10). Treatments: fed daily (control), fasted (fast), fasted for 14 days followed by 14 days of daily feeding (refed).

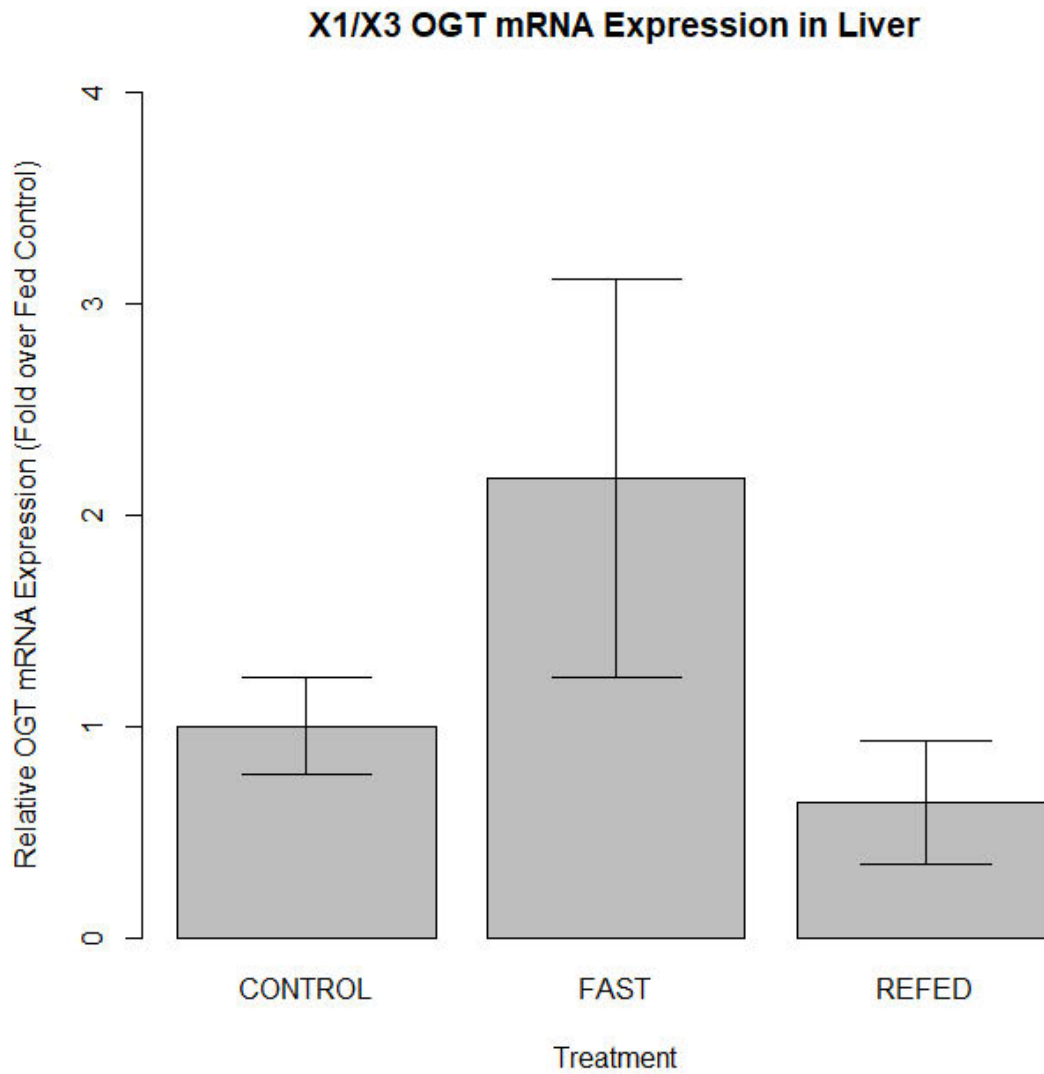


Figure 12: Changes in body weight (g) of juvenile channel catfish assigned to feeding treatments (LS Means \pm SEM; n= 8 fish per tank, 4 tanks per treatment; *p<0.05, **p<0.01). Treatments: fed once daily (control), fed twice daily (overfed), fed every other day (underfed).

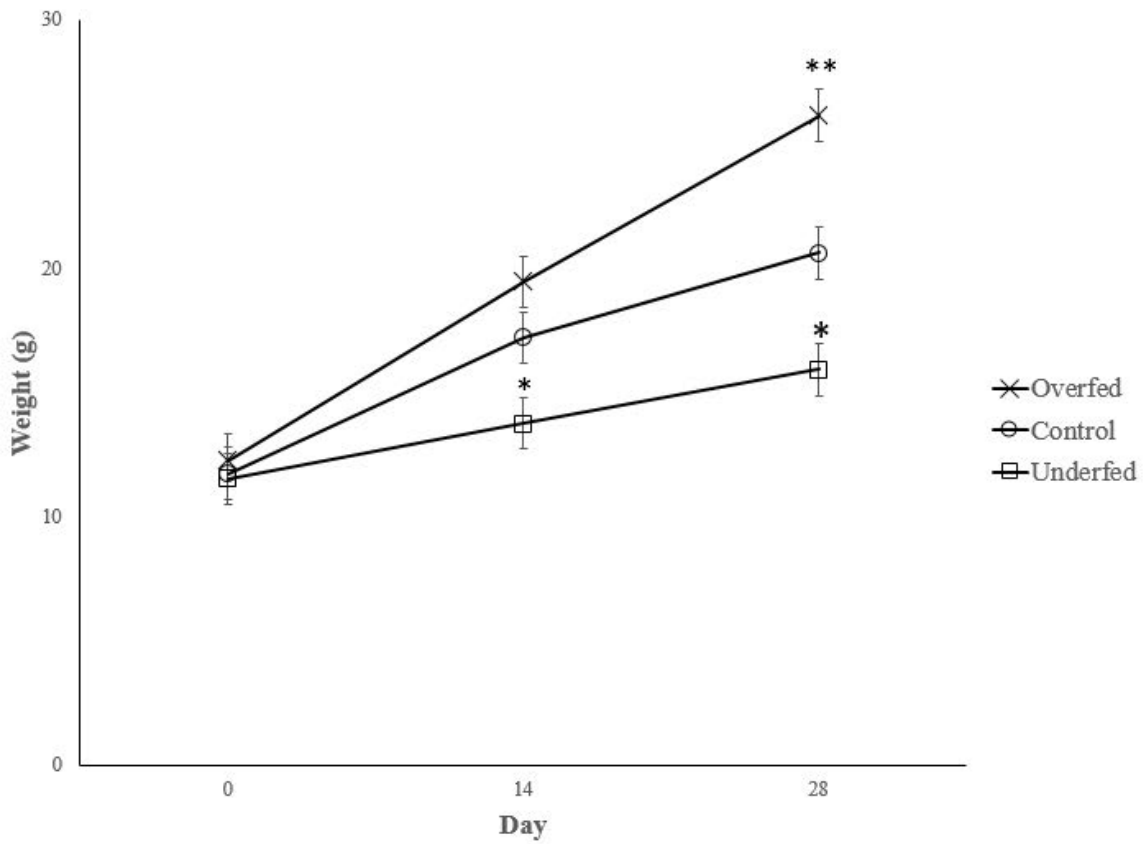


Figure 13: Relative expression of total OGT in the brain of channel catfish after 28 day feeding treatment (relative expression \pm SD; n=2 fish per tank, 4 tanks per treatment; p>0.10). Treatments: fed once daily (control), fed twice daily (overfed), fed every other day (underfed).

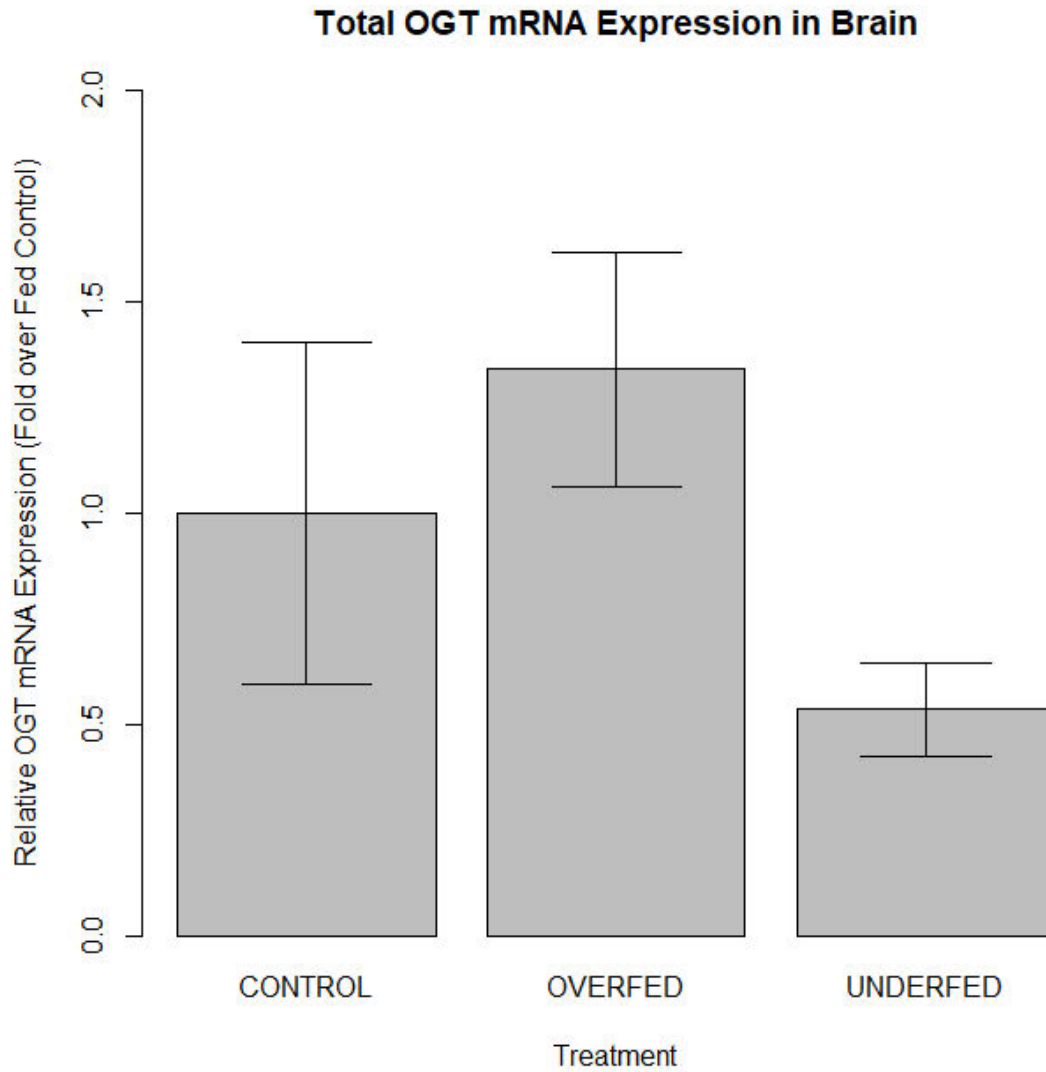


Figure 14: Relative expression of X1/X3 OGT in the brain of channel catfish after 28 day feeding treatment (relative expression \pm SD; n=2 fish per tank, 4 tanks per treatment; $p>0.10$). Treatments: fed once daily (control), fed twice daily (overfed), fed every other day (underfed).

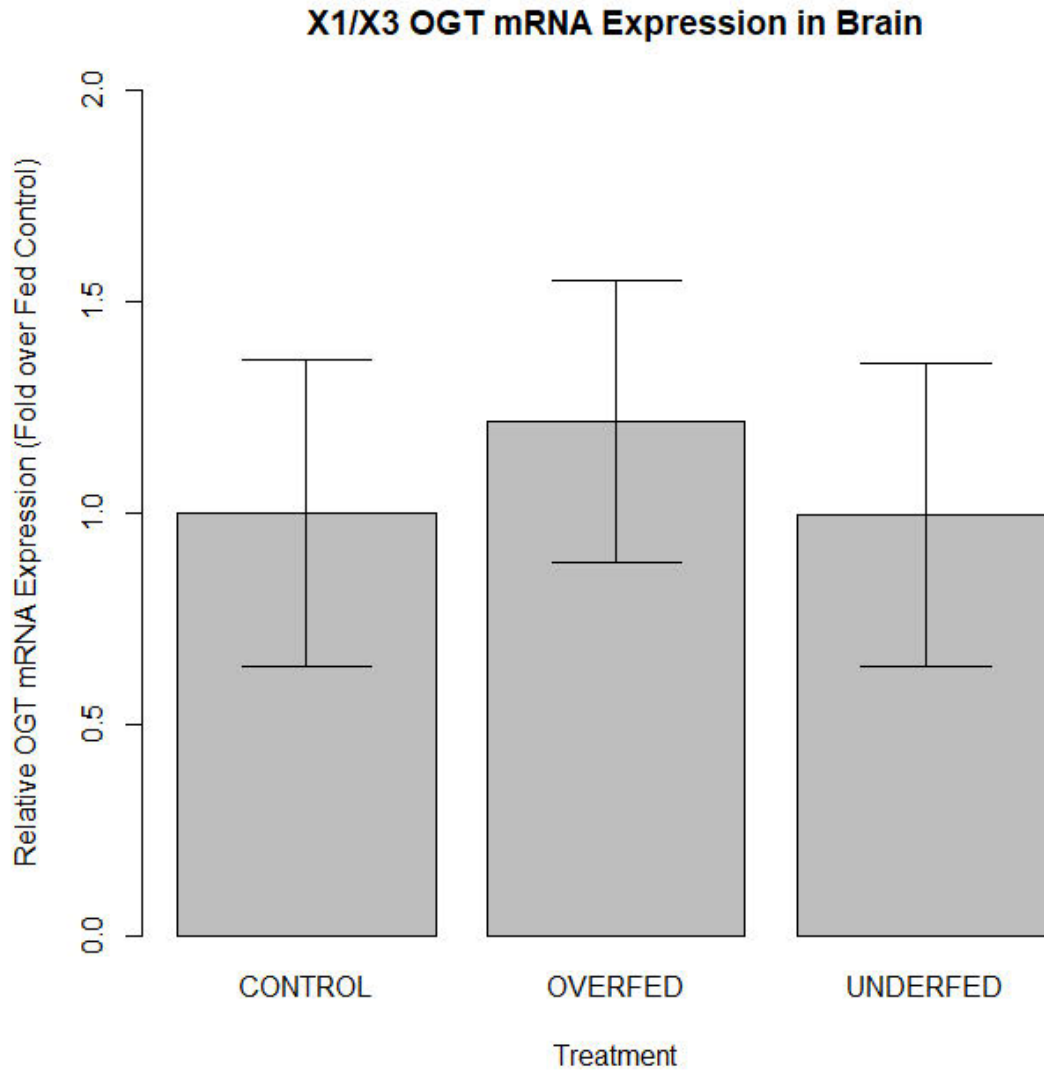


Figure 15: Relative expression of total OGT in the liver of channel catfish after 28 day feeding treatment (relative expression \pm SD; n=2 fish per tank, 4 tanks per treatment; p>0.10). Treatments: fed once daily (control), fed twice daily (overfed), fed every other day (underfed).

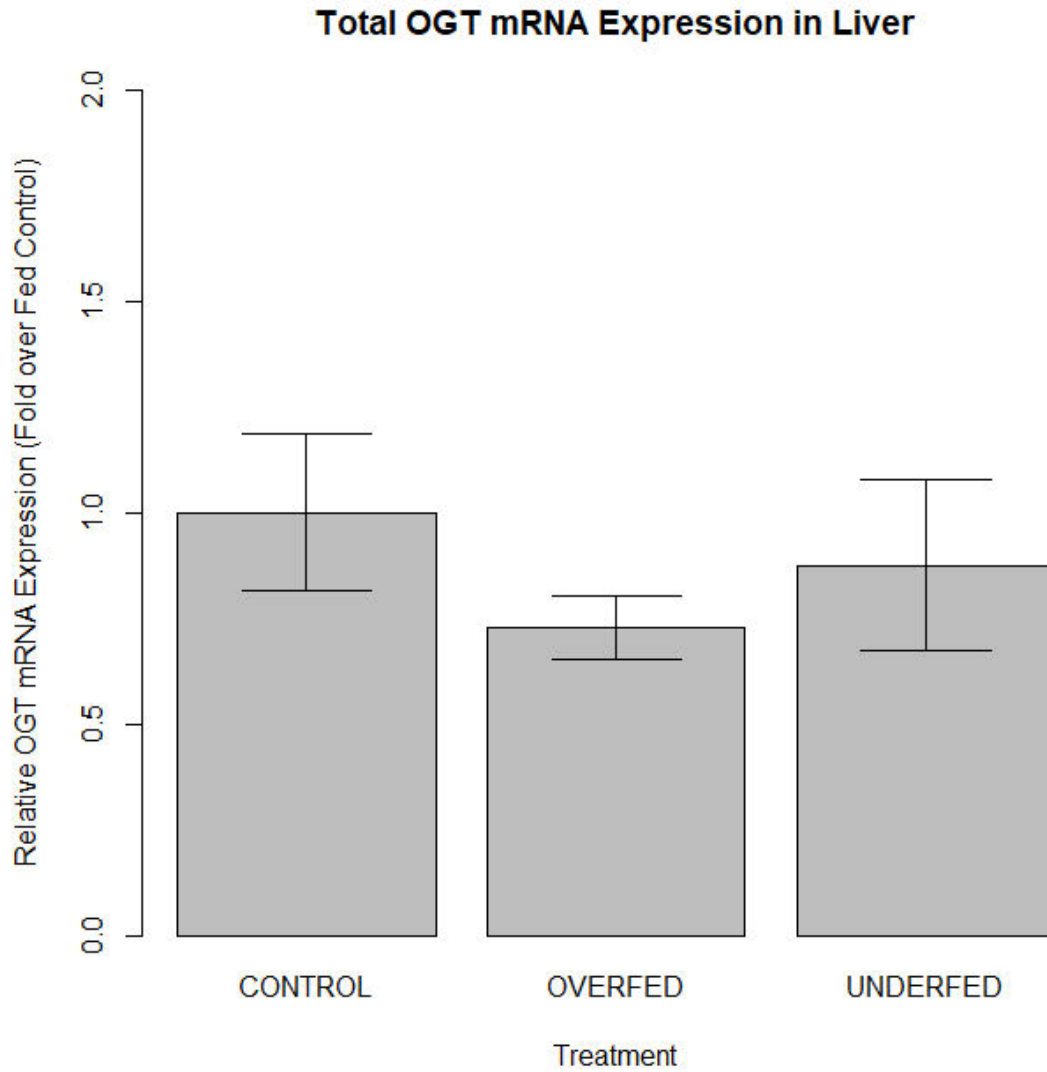
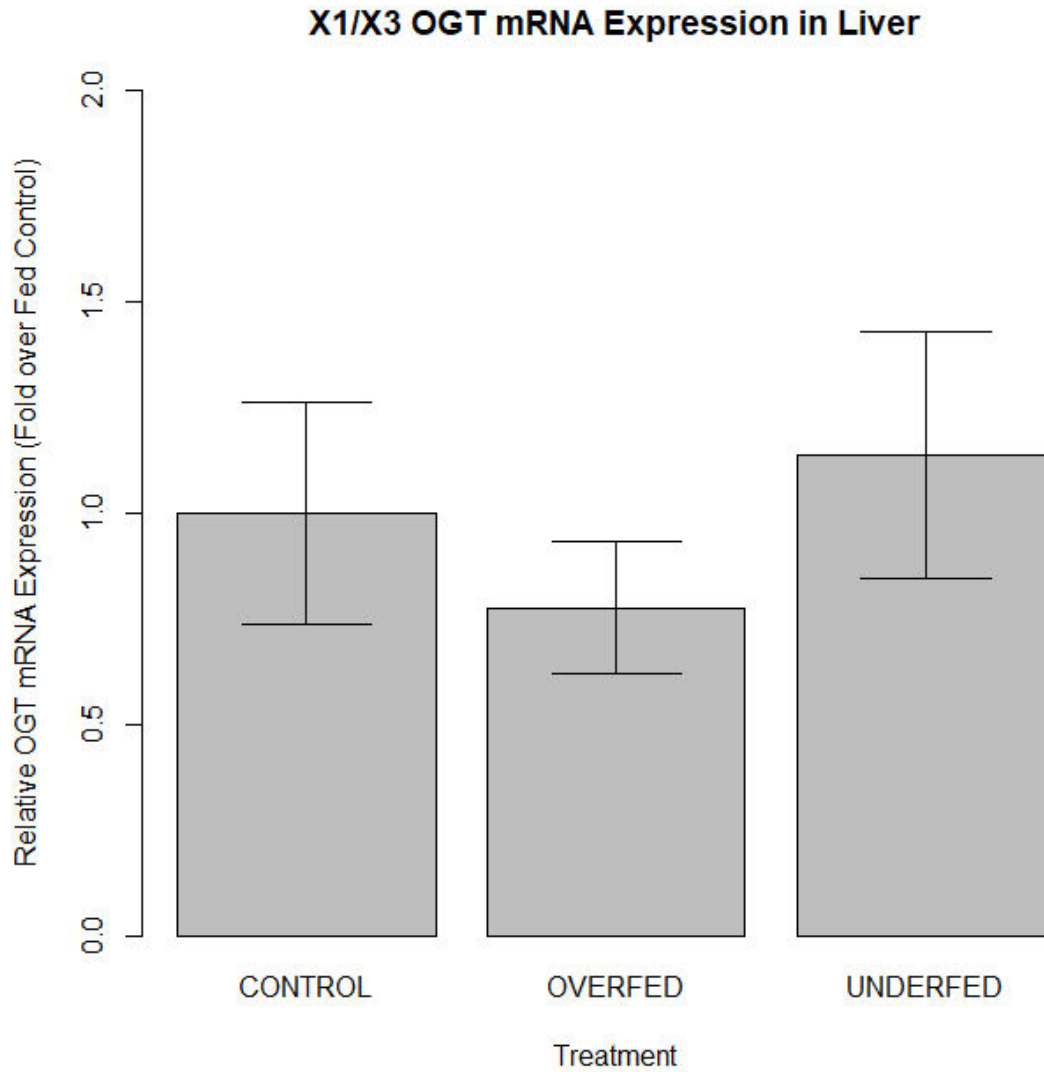


Figure 16: Relative expression of X1/X3 OGT in the liver of channel catfish after 28 day feeding treatment (relative expression \pm SD; n=2 fish per tank, 4 tanks per treatment; $p>0.10$). Treatments: fed once daily (control), fed twice daily (overfed), fed every other day (underfed).



Appendix A: Institutional Animal Care and Use Committee (IACUC) approval.



**FORT HAYS STATE
UNIVERSITY**

Forward thinking. World ready.

GRADUATE SCHOOL

Institutional Animal Care and Use Committee

To: Dr. Yasuhiro Kabayashi

From: L. Paige, Institutional Animal Use and Care Committee Administrator



Re: 18-0001 *Role of central glucose and fatty acid transport system on regulation of food intake in channel catfish*

September 8, 2017

The Institutional Animal Care and Use Committee has reviewed your IACUC protocol application titled: *Role of central glucose and fatty acid transport system on regulation of food intake in channel catfish*

and determined it to be in compliance with all USDA and PHS regulations and requirements and approved.

This approval is for the number and species of animals you listed in the protocol. Your approval will be in effect until September 7, 2021.

Please note that the IACUC is required to review and approve, prior to initiation, proposed modifications to an approved protocol.

All approved research protocols must be updated annually, and must be reviewed by the IACUC every three years. All teaching activities using vertebrate animals are reviewed annually.

IACUC approved activities may be subject to further review and approval by university officials; however, those officials may not approve an activity involving the care and use of animals if it has not been approved by the IACUC.

The Principal Investigator is responsible for following federal guidelines and university policies and procedures regarding the care and use of animals.

Please feel free to contact me if there are any questions or concerns regarding the committee's decision on your IACUC protocol.