Curcumin in diet modulate central fatty acid levels and mRNA expression of appetite regulating neuropeptides and enhances growth via GH-GHR-IGF axis in tilapia, Oreochromis mossambicus

Sruthi MV (mvsruthi930@gmail.com)
Central University of Kerala
Divya Lekha
Central University of Kerala

Research Article

Keywords: Curcumin, Tilapia, Fatty acids, Growth, Neuropeptides

Posted Date: July 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1822711/v1

Version of Record: A version of this preprint was published at Journal of Fish Biology on March 31st, 2023. See the published version at https://doi.org/10.1111/jfb.15390.

Abstract

Curcumin in 0.5 and 1% doses were given as a feed additive to tilapia (*Oreochromis mossambicus)* for 100 days to evaluate the effect of curcumin on fatty acid levels in the brain, appetite and growth axis-related gene expressions. A total of 180 fish were randomly stocked into 650 L tanks and fed with basal feed during acclimatization. Three treatment groups were established with each having three replicates and each replicate had 20 fish. They were fed twice on the experimental diets of 10% body weight ration per fish. Gas chromatography analysis revealed a significant change in the amount of total saturated fatty acids (SFAs) and total monounsaturated fatty acids (MUFAs) in the tilapia brain. The present study indicated an increase in n-3 (omega-3) and n-6 (omega-6) polyunsaturated fatty acids (PUFAs) in the brain. Real-time quantification of appetite and growth-related gene expressions revealed a significant modulation in their mRNA expressions.

Introduction

All physiological processes including feeding and growth in fish are regulated by complex mechanisms in response to various exogeneous and endogeneous signals. Among the external signals diet is the most important signal that governs fish growth. Specific diet acts on endocrine cells and regulates the expression and secretion of appetite and growth-regulating hormones in fish (Bertucci et al. 2019). Curcumin, the polyphenolic phytochemical from *Curcuma longa* Linn., has confirmed its safe and growth-promoting effects as a dietary supplement in multiple fish species (Midhun et al. 2016; Mahmoud et al. 2017; Sruthi et al. 2018; Xavier et al. 2020; 2021; Li et al. 2022).

The brain is the signaling integration center that receives, processes and responds to various internal signals (both central and peripheral) ultimately regulating growth in fish. The growth hormone (GH)-growth hormone receptor (GHR)-insulin-like growth factor (IGF) growth axis plays a critical role in the endocrine regulation of fish growth. Growth hormone, secreted from the anterior pituitary of the brain promotes cell division and somatic growth by binding to growth hormone receptor (GHR) and indirectly through the stimulation of insulin-like growth factor 1 and 2 (IGF1 and 2) in target tissues such as muscle (Bowers et al. 1984; Brooks and Waters 2010). The downstream signaling cascade promotes protein synthesis increasing muscle cell growth and differentiation which is of great importance in aquaculture (Yang et al. 2021).

In addition to the GH-GHR-IGF axis, fish growth is regulated by the expression and release of several appetite-regulating peptides. The brain (central) and the gastrointestinal tissues (peripheral) are the vital organs that are involved in the release of appetite-regulating peptides. The key central neuropeptides that stimulate (orexigenic) or inhibit (anorexigenic) feeding and thereby regulate energy intake, expenditure and storage are neuropeptide Y (NPY), agouti-related peptide (AgRP), cocaine and amphetamine-related transcript (CART) and pro-opio melanocortin (POMC) (Soengas 2021). NPY (36kDa amino acid peptide) is the most abundant neuropeptide within the brain (Loh et al. 2015). It has been shown to play an essential role in improving growth in fish by stimulating the release of GH (Li et al. 2017). The

melanocortin system includes AgRP and POMC expressing neurons. AgRP is a 128 amino acid neuropeptide and is an endogenous antagonist of melanocortin receptors 3 and 4 (MC3R and MC4R). This orexigenic factor is mainly expressed in the brain of fish (Volkoff 2016). The anorexigenic neurons in the arcuate nucleus synthesize pro-opiomelanocortin (POMC) a 267 amino acid peptide that decreases food intake and increases energy expenditure. These two neuropeptides integrate the information from circulating molecules and signals from the brain and regulate feeding (Volkoff 2016). Ghrelin is released primarily from the peripheral tissues (Kaiya et al. 2003; Londraville et al. 2014). The growth hormone (GH)-secretagogue, ghrelin, is a gut-brain 28 amino acid peptide hormone, primarily released by the stomach and binds to the growth hormone secretagogue receptor (GHSR) (Delporte 2013; Sanchez-Bretano et al. 2015). Low levels of ghrelin are also detected in the brain (Unniappan and Peter 2005; Xu and Volkoff 2009). Ghrelin also acts at the GHSR to stimulate the secretion of GH in mammals (Sun et al. 2004).

The production of appetite-regulating neuropeptides from the respective neurons occurs as a result of integrating various information including the levels of hormones and nutrients such as fatty acids, sugars and amino acids emphasizing the link between nutrient levels and the expression of neuropeptides, both are important for fish growth (Delgado et al. 2017). Fish are capable of sensing and responding to the levels of circulating metabolites such as fatty acids, sugars and amino acids (Ogunnowo-Bada et al. 2014; Efeyan et al. 2015). In fish, lipids and their constituent fatty acids are the favored source of metabolic energy for better growth and development. The fish brain is rich in fatty acids (Sargent et al. 2003). Fatty acids provide metabolic energy through mitochondrial β-oxidation in fish (Londraville and Sidell 1990; Sidell et al. 1987). Fish is an excellent, rich and unique dietary source of long-chain polyunsaturated fatty acids (LC-PUFAs), critically important for human health (Tacon and Metian 2013; Tocher 2015; Connor 2000). Eicosapentaenoic (EPA; C20:5) and docosahexaenoic (DHA; C22:6) acids, belong to the n-3 (omega-3) series of LC-PUFAs, are abundant in fish. The n-6 (omega-6) series of LC-PUFAs include linoleic (LA; C18:2) and arachidonic (AA; C20:4) acids (Connor 2000). Improving the level of LC-PUFAs is paramount and significant in aquaculture.

The present study aimed to assess the effect of curcumin-induced changes on fatty acid levels and the mRNA expression of appetite-regulating peptides and growth-regulating factors hence the GH-GHR-IGF growth axis in a commercially important fish species such as tilapia (*Oreochromis mossambicus*).

Materials And Methods

Fish collection and diet preparation

Tilapia (*O. mossambicus*), were provided by Krishi Vigyan Kendra, Kasaragod, India. Before the initiation of the experiment, they were housed in 650 L stock tanks with aerated water (26–28ºC), natural photoperiod (12 L:12 D) and fed *ad libitum* once daily with basal feed for a month. The ingredients in the basal feed were fishmeal, groundnut oil cake, rice bran and tapioca flour, with an adequate amount of vitamin drops (A-Z drops, ALKEM Laboratories Ltd., Mumbai) in the form of dry pellets (Hardy 1980;

Johnson 2004). The dried feeds were sealed in vacuum-packed bags and stored at -20ºC until further use. The proportion of ingredients and their proximate composition in the basal feed is as described earlier (Johnson 2004; Midhun et al. 2016). The lipid source in the control and experimental feeds were kept constant. All the experiments conducted were approved by the Institutional Bio-Safety and Animal Ethical Committee of the Central University of Kerala.

Experimental design

At the initiation of the experiments, fish (n = 180, individually weighed) with an average initial weight (2.82 ± 0.04 g) were distributed into three experimental groups (three replicate tanks [0.61x0.30x0.30 m] series each) of control, and two curcumin-treated groups. Tank A series constituted the control group, and B and C series 0.5 and 1% curcumin, respectively. The experimental feed pellets were prepared by supplementing 0.5 and 1% curcumin (by weight) to the basal feed and air-dried in dark. The doses of 0.5 and 1% curcumin were selected on the basis of previous studies (Midhun et al. 2016; Sruthi et al. 2018). The fish were fed twice daily in equal proportions (8.00 a.m-9.00 am and 4.00 p.m-5 pm) on the experimental diet of a 10% body weight ration per fish, for a period of 100 days. The weight of the fish were checked every twenty days of feeding, and the feed given was modified according to the body weight. During the experiment, each tank was individually aerated and the water was refreshed daily one-third of the total volume. The feeding trial was completed under natural photoperiod.

Experimental sampling

At the termination of 100 days, the fish were starved overnight. The following day they were randomly chosen from each tank (n = 10) and anesthetized using tricaine methane sulfonate (MS-222, Sigma, USA). The fish were then decapitated, using sterilized scissors and surgical blades. The samples for RNA isolation (brain, stomach and muscle) were excised from the fish and immediately transferred into 1mL Tri reagent (Sigma-Aldrich). Brain and muscle samples were excised from the fish and were transferred to a -80ºC freezer until further analysis (Panasonic ultra-low temperature freezer, Japan).

Analysis of fatty acid composition by Gas Chromatography

The extraction of fat in the brain was done with chloroform/methanol (2:1 v/v) (Folch et al. 1957). Lipid samples were converted to their constituent fatty acid methyl esters (FAMEs) following the method of Metcalfe et al. (1966). Analysis of methyl esters was performed by a capillary gas chromatograph model Agilent 6890 (Agilent Technology, USA) equipped with a split-splitless injector, flame ionization detection (FID) system used to separate and quantify each FAME component. Compounds were identified by comparison of the retention times of 37 components FAME mix 47885-U (Supelco, Germany).

Health lipid index and PUFA damage

The lipid quality index was calculated according to Ulbricht and Southgate (1991). Thrombogenic Index (TI) is the relationship between the pro-thrombogenetic (SFAs) and the anti-thrombogenetic (MUFAs and PUFAs) fatty acids.

The Thrombogenic Index was calculated as follows:

TI = [(C14 + C16 + C18)/ (0.5 x Sum MUFAs + 0.5 x Sum n-6 PUFAs + 3 x Sum n-3 PUFAs + (n-3/n-6))].

The Polyene index (PI) is used to measure the PUFA damage.

Polyene index was calculated according to Lubis and Buckle (1990).

PI= [(C20:5 + C22:6)/C16].

Quantitative RT - PCR Gene Expression Measurement

The total RNA isolation from the brain, stomach and muscle was done by the Trizol method. The concentration and quality of RNA were validated spectrophotometrically using a Nanodrop (Thermoscientific, USA) by OD 260:280 ratios. One microgram of RNA from each sample was reverse transcribed to cDNA (Thermoscientific, USA). Real-time quantification of genes was performed using SYBR Green Supermix (Biorad). The primer sequences specific for each gene (Table 1) were prepared according to the IDT chart sheet (Integrated DNA Technologies, USA). The amplification of genes was performed in a 96-well plate loaded with10µL SYBR, 1µL reverse, 1µL forward primers and 2µL cDNA in each well. The RT-qPCR cycling programme consisted of an initial step of 95^0C for 10s, followed by 40 cycles of 95^0C for the 30s and specific annealing and extension temperatures for 15s (Table 1). RT-qPCR was performed using LC 480 RTPCR (Roche, USA). Among the experimental groups, β-actin gene expression was unchanged and is used as a reference gene. The mRNA expression levels were calculated according to the 2^{-DDCT} method (Livak and Schmittgen 2001). The mRNA expression in experimental groups was calculated as a fold of the mRNA expression in control, which was considered to be 1. Triplicate readings were taken with appropriate non-template control (NTC). At the end of each run, a melting curve analysis was performed to confirm the specificity of the amplified product.

Statistical Analysis

The SPSS version 16 was used to analyse all data. One-way ANOVA was used for the analysis of the data. The statistical significance is set at $p \leq 0.05$ in Duncan's multiple range test (Duncan 1995).

Results

Brain fatty acids

The fatty acid composition in brain of tilapia fed different doses of curcumin is presented in Table 2. Gas chromatography confirmed the identification and quantification of 25 fatty acids in the brain of tilapia. Palmitic acid (C16), myristoleic acid (C14:1), elaidic acid (C18:1), docosahexaenoic acid (C22:6) and linoelaidic acid (C18:2) were recorded in the highest proportions. Compared to control, the total amount of SFAs and PUFAs in the brain significantly increased with both doses of curcumin ($p \leq 0.05$). The total amount of MUFAs significantly decreased with two doses of curcumin in the tilapia brain ($p \leq 0.05$). The

main LC-PUFAs detected were DHA, LA and AA. The n-3 PUFAs such as EPA and DHA and n-6 PUFAs such as LA and AA were increased significantly in the brain with 0.5 and 1% curcumin supplementation compared to control ($p \leq 0.05$).

Nutritional quality, health lipid index and PUFA damage

Table 2 shows different ratios of fatty acids related to nutritional quality in the brain of tilapia fed different doses of curcumin. The ratios, n-3/n-6 and DHA/EPA were significantly increased with different doses of curcumin-fed tilapia compared to control ($p \leq 0.05$). The PUFAs/SFAs ratio in the brain was significantly increased in both curcumin-fed groups ($p \leq 0.05$). Different doses of curcumin significantly increased the PUFAs/n-3 ratio in the brain ($p \leq 0.05$). Health lipid index and PUFA damage in the brain of tilapia fed different doses of curcumin are represented in table 3. The index of thrombogenicity values was significantly decreased in 0.5 and 1% curcumin-fed groups ($p \leq 0.05$). The polyene index values were significantly increased in both curcumin groups ($p \leq 0.05$).

Modulation of mRNA expression of appetite-regulating peptides and growth-regulating factors by curcumin

In the brain, NPY mRNA expression significantly increased with the supplementation of 0.5% curcumin ($p \leq 0.05$) (Fig. 1). Significant downregulation of AgRP, POMC, ghrelin and GHSR gene expressions in the brain was observed with 0.5 and 1% curcumin groups ($p \leq 0.05$) (Figs. 2, 3, 4 and 5 respectively). A significant downregulation in stomach ghrelin and its receptor gene expressions was observed with 0.5 and 1% curcumin groups ($p \leq 0.05$) (Figs. 6 and 7 respectively). The fish fed with 0.5 and 1% curcumin groups showed a significant increase in GHR, IGF1 and IGF2 (Figs. 8,9 and 10 respectively) gene expressions in the muscle of tilapia ($p \leq 0.05$).

Discussion

Lipids and their constituent fatty acids are the major sources of metabolic energy in fish. Fatty acids are involved in the structure of most lipids and are important in the structure of cell membranes and also function in intracellular signal transduction and metabolic pathways thereby playing significant roles in fish growth (Tocher 2003). The fatty acid composition of the fish is dependent upon the interaction of diet with endogenous metabolism and can be improved through nutrition (Tocher and Glencross 2015). In the present study, both 0.5 and 1% doses of curcumin significantly increased the amount of total SFAs and PUFAs in the brain of tilapia when compared to control. A significant decline in the total amount of MUFAs was also reported with two doses of curcumin in the tilapia brain. Compared to control both doses of curcumin supplementation significantly elevated the levels of LC-PUFAs such as EPA, DHA LA, and AA in the brain. The content of DHA was more than EPA in experimental groups. This is because, relative to DHA, EPA shows higher beta-oxidation (Luo et al. 2010). The higher catabolism of EPA compared to DHA occurs may be to meet the requirement for cell membrane composition and the complex function of brain tissues. Studies in tilapia showed an elevated amount of LC-PUFAs due to the efficient conversion of fatty acids to n-3 LC-PUFAs (Teoh et al. 2011). In the present study, the higher values of DHA, a major n-3 LC-PUFA, recorded in tilapia fed after curcumin supplemented diet implies that

tilapia may have the ability to increase the levels of LC-PUFAs by converting SFAs and EPA to LC-PUFAs. Moreover, Wu et al. (2015) have reported in mammals that, the elevated DHA level was associated with the elevated levels of the enzymes, fatty acid desaturase 2 (FADS2) and elongase 2, two important enzymes for the biosynthesis of DHA. In male Sprague-Dawley rats, curcumin activated these enzymes that may enhance the synthesis of DHA from ALA in brain tissue (Wu et al. 2015). The role of curcumin in the upregulation of peroxisome proliferator-activated receptor-alpha (PPAR-α), a member of the nuclear hormone receptor family, in Sprague-Dawley rats suggests its role in the metabolism of fatty acids (Zhao et al. 2017). However, no similar studies were documented in lower vertebrates like fish for comparison.

Curcumin supplementation in the diet significantly improved the nutritional value of tilapia, an important food fish. The n-3/n-6 ratio in fish is a good index for evaluating the nutritional value. The higher ratios are associated with the prevention of coronary heart disease and reduced cancer risk, autoimmune disorders, allergies and some mental disorders (Kinsella 1990). Our study reports for the first time that, curcumin supplementation in the tilapia diet significantly increased the n-3/n-6 ratio in the brain, indicating the enhancement of the nutritional value. Similarly, both doses of curcumin significantly increased the PUFAs/SFAs ratio when compared to control. The ratio was higher than that of 0.45, considered beneficial for human health as it improves protection from coronary heart diseases (FAO 1994; Simat et al. 2015). The index of thrombogenicity serves as an indicator of lipid quality and measures the global dietetic quality of lipids and their potential effect on heart diseases (Sabikhi 2004). It reflects the tendency to form clots in the blood vessels or the probability of increasing the incidence of thrombus formation and accounts for the different effects that a single fatty acid has on human health (Garaffo et al. 2011). This study revealed that different doses of curcumin significantly decreased TI levels in the brain of tilapia. The polyene index is used to measure the PUFA damage. The lower PI indicates that the PUFA being damaged (Simat et al. 2015). Different doses of curcumin in the feed significantly increased the levels of PI in tilapia in this study. This revealed that the PUFA in the brain had not been damaged.

The supplementation of both doses of curcumin significantly modulated the expression of appetite-regulating neuropeptides such as NPY, AgRP and POMC in the brain of tilapia. The mRNA expression of ghrelin and its receptor in the brain was significantly decreased by 0.5 and 1% curcumin. The production of neuropeptides from the respective neurons occurs as a result of integrating various information including the levels of lipids and their constituent fatty acids (Delgado et al. 2017). Fish have the ability for sensing and responding to the levels of specific nutrients including lipids and their constituent fatty acids signifying the existence of nutrient-sensing mechanisms in the fish (Ogunnowo-Bada et al. 2014; Efeyan et al. 2015). Fatty acid sensing in the fish brain is the ability of a specialized cell to detect and respond to the levels of fatty acids. The sensors of fatty acids in the brain of fish regulate energy intake, expenditure and homeostasis by sensing the levels of LC-PUFAs *via* different mechanisms. Sensing a change in the levels of fatty acid can directly or indirectly stimulate the neuronal circuits in the brain and result in the expression of orexigenic and anorexigenic neuropeptides (Conde-Sieira and Soengas 2017). Available studies suggest that activation of the nutrient-sensing system inhibits AMP-activated protein kinase (AMPK) and activates protein kinase B (Akt) and the mechanistic target of rapamycin (mTOR).

The downstream signal transduction regulates the phosphorylation of transcription factors. Changes in the phosphorylation of transcription factors such as cAMP response element-binding protein (CREB), forkhead boxo1 (FOXO1) and brain homeobox transcription factor (BSX) control the expression of NPY/AgRP and POMC/CART resulting in the regulation of feed intake hence the growth (Soengas 2021). In the present study curcumin in the feed may modulate the levels of fatty acids such as EPA and DHA that may stimulate the nutrient-sensing system thereby causing a downstream signaling cascade and the subsequent production of neuropeptides ultimately regulating anorectic response in tilapia. The regulation in feed intake (FI) by curcumin in tilapia is further supported by our data in which the FI was significantly reduced by curcumin at 0.5 and 1% doses when compared to control (Sruthi et al. 2018). This is the first long-term *in vivo* study in tilapia demonstrating the effect of curcumin and fatty acids on the expression of neuropeptides in the brain leading to a change in feed intake response.

The NPY gene expression significantly increased with the supplementation of 0.5% curcumin in the brain. NPY plays a pivotal role in the regulation of the GH-GHR-IGF growth axis. Studies showed the increase in the mRNA expression of NPY can be correlated with an increase in serum GH levels (Riley et al. 2009). Further, *in vitro* and *in vivo* studies in goldfish emphasize that NPY stimulates GH secretion (Peng et al. 1993). Studies in grass carp revealed the up regulation of hepatic IGF mRNA expression following NPY injection (Zhou et al. 2013). This is in accordance with the results in which there was an upregulation of hepatic IGFs by curcumin in tilapia which coincides with the enhanced NPY mRNA expression (Sruthi et al. 2018). Significant downregulation of AgRP and POMC expression in the brain was observed with 0.5 and 1% curcumin groups when compared to control. It should be noted that their function may differ among species or that they may even have multiple functions depending on their interactions with other systems. Further investigations could explore the exact mechanism for the regulation of neuropeptides by curcumin since no studies were available for comparison.

The gut-brain peptide hormone ghrelin, regulate a vast array of biological processes in fish by binding to ghrelin receptors (Sanchez-Bretano et al. 2015). Herein, we report for the first time that, there is a significant decrease in the brain and stomach mRNA expression of ghrelin and its receptor, GHSR, in both 0.5 and 1% curcumin-fed tilapia. Ghrelin is crucial in the counter-regulation of GH (Nass et al. 2010). The physiological role of ghrelin is that it orchestrates GH secretion (Zhao 2010). We have also reported that dietary curcumin significantly increased the expression of GH in the brain of tilapia (Sruthi et al. 2018). Collectively, these results implied a major finding regarding the ghrelin feedback system, in which, increased GH expression may negatively feedback and possibly downregulate ghrelin expression in the brain and stomach. The negative feedback action of GH on the stomach was seen to increase stomach ghrelin production and secretion in aged rats in response to reduced GH in circulation (Qi et al. 2003). Together, these findings strengthen the fact that, there exists a stomach/ghrelin-pituitary/GH endocrine axis, and that, changes in GH regulate stomach ghrelin expression. Studies in mammals, birds, amphibians and teleost fish have reported that ghrelin binds to GHSR and stimulates the release of GH (Kojima et al. 1999; Kaiya et al. 2001, 2002; Unniappan and Peter 2004). The gene expression of GHSR is affected by various hormonal factors, it is inhibited by GH and IGF1 (Kamegai et al. 2005). Earlier studies

in tilapia revealed that ghrelin stimulates GH release from the tilapia pituitary, increased hepatic IGF1 and GHR mRNA expressions in tilapia (Fox et al. 2007).

Significant increases in muscle GHR, IGF1 and 2 genes were observed in tilapia fed with 0.5 and 1% doses of curcumin. Our previous studies showed that curcumin at 0.5 and 1% doses in tilapia significantly upregulated the mRNA expressions of GH in brain and IGF1 and 2 in muscle in short term (35 days) (Midhun et al. 2016). Also, curcumin at different doses significantly up regulated the mRNA expressions of GH in brain and IGF1 and 2 in the liver in long term in tilapia (100 days) (Sruthi et al. 2018). It is well known that the somatic growth in fish and mammals is orchestrated by the GH-GHR-IGF axis (Velez and Unniappan 2021). GH acts on somatic cells by binding to GHR and augmenting protein synthesis, amino acid uptake and release of IGF1 and 2 resulting in enhanced somatic growth (Humbel 1990). The growth of fish is directly linked to muscle growth which in turn is regulated by IGFs. Curcumin may increase the gene expression of GHR and may cause an elevation in ligand sensitivity to GH-induced IGF production in muscle. The up regulation of IGF genes in muscle may result in the increase in growth parameters as indicated by the increased total weight gain (TWG, g), specific growth rate (SGR, %) and reduced feed conversion rate (FCR, Kg/Kg) reported in our earlier findings (Sruthi et al. 2018).

Information on the beneficial role of curcumin in the regulation of fatty acid levels and the expression of appetite-regulating neuropeptides and growth regulating factors will contribute more to research on feed intake and growth in fish. Increased LC-PUFAs in the tilapia brain after dietary curcumin supplementation is of great importance to meet an efficient and economical supply for the production of LC-PUFAs enriched food products and to expand their markets due to the benefits to these LC-PUFAs in preventing cardiovascular disease. Consumption of nutrient-enhanced tilapia will prevent disease incidence and improve the health status of people. Hence, the quality and quantity of aquaculture products can be improved by using a safe, viable and environmentally oriented aquafeed supplementation such as curcumin.

Declarations

Ethical Approval

All the experimental protocols performed in this study were approved by Institutional Bio Safety and Animal Ethical Committee, Central University of Kerala.

Acknowledgements

The authors would like to thank Central University of Kerala, Prof. Dr. Oommen V Oommen, Honorary Professor, Department of Computational Biology and Bioinformatics, University of Kerala, Synthite Industries, Kerala, Kerala State Council for Science Technology and Environment, Thiruvananthapuram, for Senior Research Fellowship.

Competing interests

The authors declare that we do not have any competing interests in the matter stated in this paper. All the authors approve on the presented material of this paper.

Author's contribution

S.M.V performed majority of the work, analysed and interpreted the data, drafted the manuscript, D.L. conceptualized, designed the experiment, analysed and interpreted the data, reviewed, edited and supervised the entire work. All authors read and approved the final manuscript.

Funding

This work was supported by Kerala State Council for Science Technology and Environment (KSCSTE). S.M.V. has received research support from KSCSTE.

Availability of data and materials

The datasets generated during the current study are not publically available but are available from the corresponding author on reasonable request.

References

1. Bertucci JI, Blanco AM, Sundarrajan L, Rajeswari JJ, Velasco C, Unniappan S. 2019. Nutrient regulation of endocrine factors influencing feeding and growth in fish. Front. Endocrinol. https://doi.org/10.3389/fendo.2019.00083
2. Bowers CY, Momany FA, Reynolds GA, Hong A. 1984. On the *in vitro* and *in vivo* activity of a new synthetic hexapeptide acts on the pituitary to specifically release growth hormone. Endocrinology. 114, 1537-45.
3. Brooks AJ, Waters MJ. 2010. The growth hormone receptor: mechanism of activation and clinical implications. Nat. Rev. Endocrinol. 6, 515-25.
4. Conde-Sieira M, Soengas JL. 2017. Nutrient sensing systems in fish: Impact on food intake regulation and energy homeostasis. Front. Neurosci. https://doi.org/10.3389/fnins.2016.00603
5. Connor, WE. 2000. Importance of n-3 fatty acids in health and disease. Am. J. Clin. Nutr. 71, 171S-5S.
6. Delgado MJ, Cerda-Reverter JM, Soengas JL., 2017. Hypothalamic integration of metabolic, endocrine and circadian signals in fish: Involvement in the control of food intake. Front. Neurosci. https://doi.org/10.3389/fnins.2017.00354
7. Delporte C. 2013. Structure and physiological actions of ghrelin. Scientifica. 1 - 25.
8. Duncan DB. 1955. Multiple range and multiple F test. Biometrics. 11, 1-42.
9. Efeyan A, Comb WC, Sabatini DM. 2015. Nutrient sensing mechanisms and pathways. Nature. 517, 302–310. doi: 10.1038/nature14190
10. FAO/WHO. 1994. Fats and oils in human Nutrition: Report of a joint expert consultation.

11. Folch J, Lees M, Sloanestanley GH. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497-509.
12. Fox BK, Breves JP, Hirano T, Grau EG. 2009. Effects of short and long term fasting on plasma and stomach ghrelin, and the growth hormone/insulin like growth factor1 axis in the tilapia, *Oreochromis mossambicus*. Domest. Anim. Endocrinol. 37, 1-11.
13. Garaffo MA, Vassallo-Agius R, Nengas Y, Lembo E, Rando R, Maisano R, Dugo G, Giuffrida D. 2011. Fatty acids profile, atherogenic (IA) and thrombogenic (IT) health lipid indices, of raw roe of blue fin tuna (*Thunnus thynnus* L.) and their salted product "Bottarga". Sci. Res. 2, 736-743.
14. Hardy R. 1980. Fish feed formulation. In: Lectures Presented at the FAO/UNDP Training Course in Fish Feed Technology. 1980. University of Washington, Seattle, pp. 233–240.
15. Humbel RE. 1990. Insulin like growth factors 1 and 2. Eur. J. Biochem. 190. 445-462.
16. Johnson C. 2004. Influence of certain plant extract on growth and metabolism of teleosts (*Anabas testudineus* and *Labeo rohita*) and a mammal (*Rattus norvegicus*). PhD thesis, University of Kerala. In: Thiruvananthapuram. Kerala, India.
17. Kaiya H, Kojima M, Hosoda H, Koda A, Yamamoto K, Kitajima Y, Masaru MM, Minamitake Y, Kikuyama S, Kangawa K. 2001. Bullfrog ghrelin is modified by n-octanoic acid at its third threonine residue. J. Biol. Chem. 276, 40441-40448.
18. Kaiya H, Kojima M, Hosoda H, Moriyama S, Takahashi A, Kawauchi H, Kangawa K. 2003. Peptide purification, complementary deoxyribonucleic acid (DNA) and genomic DNA cloning, and functional characterization of ghrelin in rainbow trout. Endocrinology. 144, 5215–26. doi: 10.1210/en.2003-1085
19. Kaiya H, van der Geyten S, Kojima M, Hosoda H, Kitajima Y, Matsumoto M, Geelissen S, Darras VM, Kangawa K. 2002. Chicken ghrelin: purification, cDNA cloning, and biological activity. Endocrinology. 143, 3454-3463.
20. Kamegai J, Tamura H, Shimizu T, Ishii S, Sugihara H, Oikawa S. 2005. Insulin like growth factor 1 down regulates ghrelin receptor (growth hormone secretagogue receptor) expression in the rat pituitary. Regul. Pept. 127, 203-6. doi:10.1016/j.regpep.2004.12.001.
21. Kinsella JE, Broughton KS, Whelan JW. 1990. Dietary unsaturated fatty acids: Interactions and possible needs in relation to eicosanoid synthesis. J. Nutr. Biochem. 1, 123-141.
22. Kojima M, Hosoya H, Date Y, Nakazato M, Matsuo H, Kangawa K. 1999. Ghrelin is a growth hormone releasing acylated peptide from stomach. Nature. 402, 656-660.
23. Li M, Kong Y, Wu X, Guo G, Sun L, Lai Y, Zhang J, Niu X. 2022. Effects of dietary curcumin on growth performance, lipopolysaccharide-induced immune responses, oxidative stress and cell apoptosis in snakehead fish (*Channa argus*). Aquaculture Reports, 22, 100981.
24. Li M, Tan X, Sui Y, Jiao S, Wu Z, Wang L, You F. 2017. The stimulatory effect of neuropeptide Y on growth hormone expression, food intake, and growth in olive flounder (*Paralichthys olivaceus*). *Fish Physiol Biochem* 43, 11–18. https://doi.org/10.1007/s10695-016-0263-x

25. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using realtime quantitative PCR and the 2^{-DDCT} method. Methods. 25, 402-408.
26. Loh K, Herzog H, Shi YC. 2015. Regulation of energy homeostasis by the NPY system. Trends Endocrinol. Metab. 26, 125-135.
27. Londraville RL, Macotela Y, Duff RJ, Easterling MR, Liu Q, Crespi EJ. 2014. Comparative endocrinology of leptin: assessing function in a phylogenetic context. Gen. Comp. Endocrinol. 203, 146-57. doi: 10.1016/j.ygcen.2014.02.002
28. Londraville RL, Sidell BD. 1990. Ultrastructure of aerobic muscle in Antarctic fishes may contribute to maintenance of diffusive fluxes. J. Exp. Biol. 150, 205-220.
29. Lubis L, Buckle KA. 1990. Rancidity and lipid oxidation of dried-salted sardines. Int. J. Food Sci. Tech. 25, 295-303.
30. Luo G, Xu J, Teng Y, Ding C, Yan B. 2010. Effects of dietary lipid levels on growth, digestive enzyme, feed utilization and fatty acid composition of Japanese sea bass (*Lateolabrax japonicas* L.) reared in freshwater. Aquac. Res. 4, 210-219.
31. Mahmoud AA, Al-Sagheer AA, Reda FM, Mahgoub SA, Ayyat MS. 2017. Dietary curcumin supplement influence on growth, immunity, antioxidant status and resistance to *Aeromonas hydrophila* in *Oreochromis niloticus*. Aqua. 475, 16-23.
32. Metcalfe LD, Schmitz AA, Pelka JR. 1966. Rapid preparation of fatty acid esters from lipids for gas chromatographic analysis. Anal. Chem. 38. 514-515.
33. Midhun SJ, Arun D, Edatt L, Sruthi MV, Thushara VV, Oommen VO, Sameer Kumar VB, Divya L. 2016. Modulation of digestive enzymes, GH, IGF1 and IGF2 genes in the teleost, Tilapia (*Oreochromis mossambicus*) by dietary curcumin. Aquac. Int. 24, 1277-1286.
34. Nass RM, Gaylinn BD, Rogol AD, Thorner MO. 2010. Ghrelin and growth hormone: Story in reverse. PNAS. 107, 8501-8502.
35. Ogunnowo-Bada E, Heeley N, Brochard L, Evans ML. 2014. Brain glucose sensing, glucokinase and nural control of metabolism and islet function. Diab. Obes. Metab. 16, 26–32. doi: 10.1111/dom.12334
36. Peng C, Humphries S, Peter RE, Rivier JE, Blomqvist AG, Larhammar D. 1993. Actions of goldfish neuropeptide Y on the secretion of growth hormone and gonadotropin-II in female goldfish. Gen. Comp. Endocrinol. 90, 306-317.
37. Qi X, Reed J, Englander EW, Chandrashekar V, Bartke A, Greeley GH. 2003. Evidence that growth hormone exerts a feedback effect on stomach ghrelin production and secretion. Exp. Biol. Med. 228, 1028-1032. doi: org/10.1177/153537020322800907.
38. Riley Jr LG, Walker AP, Dorough CP, Schwandt SE, Grau EG. 2009. Glucose regulates ghrelin, neuropeptide Y, and the GH/IGF1 axis in the tilapia, *Oreochromis mossambicus*. Comp. Biochem. Physiol. 154, 541-546. doi:10.1016/j.cbpa.2009.08.018.

39. Sabikhi L. 2004. Designer milk, an imminent milestone in dairy biotechnology. Curr. Sci. 87, 1530-1535.
40. Sanchez-Bretano A, Alonso-Gomez AL, Delgado MJ, Isorna E. 2015. The liver of goldfish as a component of the circadian system: integrating a network of signals. Gen. Comp. Endocrinol. 221, 213-216. doi: 10.1016/j.ygcen.2015.05.001.
41. Sargent JR, Tocher DR, Gordonbell J. 2003. The lipids. In Fish nutrition third edition, 181-257. https://doi.org/10.1016/B978-012319652-1/50005-7
42. Sidell BD, Driedzic WR, Stowe DB, Johnston IA. 1987. Biochemical correlations of power development and metabolic fuel preferenda in fish hearts. Physiol. Zool. 60, 221-232.
43. Simat V, Bogdanovic T, Poljac V, Petricevic S. 2015. Changes in fatty acid composition, atherogenic and thrombogenic health lipid indices and lipid stability of bogue (*Boops boops* L. 1758) during storage on ice: effect of fish farming activities. J. Food Comp. Anal. 40, 120-125. doi:10.1016/j.jfca.2014.12.026.
44. Soengas JL. 2021. Integration of nutrient sensing in fish hypothalamus. Front. Neurosci. https://doi.org/10.3389/fnins.2021.653928
45. Sruthi MV, Nair AB, Arun D, Thushara VV, Sheeja CC, Vijayasree AS, Oommen OV, Divya L. 2018. Dietary curcumin influences leptin, growth hormone and hepatic growth factors in tilapia (*Oreochromis mossambicus*). Aqua. 496, 105-111. doi.org/10.1016/j.aquaculture.2018.06.083.
46. Sun Y, Wang P, Zheng H, Smith RG. 2004. Ghrelin stimulation of growth hormone release and appetite is mediated through the growth hormone secretagogue receptor. Proc. Natl. Acad. Sci. USA, 101:4679-4684. doi: 10.1073/pnas.0305930101
47. Tacon AG, Metian M. 2013. Fish matters: Importance of aquatic foods in human nutrition and global food supply. Rev. Fish. Sci. 21, 22-38.
48. Teoh CY, Turchini GM, Ng WK. 2011. Genetically improved farmed Nile tilapia and red hybrid tilapia showed differences in fatty acid metabolism when fed diets with added fish oil or a vegetable oil blend. Aqua. 312, 126-136.
49. Tocher D, Glencross B. 2015. Lipids and fatty acids. In dietary nutrients, additives, and fish health, 47-94.
50. Tocher DR. 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in perspective. Aqua. 449, 94-107.
51. Tocher DR. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107-184.
52. Ulbricht TL, Southgate DAT. 1991. Coronary heart disease: Seven dietary factors. Lancet, 338, 985e992.
53. Unniappan S, Peter RE. 2004. *In vitro* and *in vivo* effects of ghrelin on luteinizing hormone and growth hormone release in goldfish. Am. J. Physiol. 286, R1093-R1101.

54. Unniappan S, Peter RE. 2005. Structure, distribution and physiological functions of ghrelin in fish. Comp. Biochem. Physiol. 140, 396-408.
55. Velez EJ, Unniappan S. 2021. A comparative update on the neuroendocrine regulation of growth hormone in vertebrates. Front. Endocrinol. 11, 614981.
56. Volkoff H. 2016. The neuroendocrine regulation of food intake in fish: a review of current knowledge. Front. Neurosci. 10, 1-31. https://doi.org/10.3389/fnins.2016.00540.
57. Wu A, Noble EE, Tyagi E, Ying Z, Zhuang Y, Gomez-Pinilla F. 2015. Curcumin boosts DHA in the brain: implications for the prevention of anxiety disorders. Biochim. Biophys. Acta. 1852, 951-961. doi:10.1016/j.bbadis.2014.12.005.
58. Xavier MJ, Dardengo GM, Navarro-Guillen C, Lopes A, Colen R, Valente LMP, Conceicao LEC, Engrola S. 2021. Dietary curcumin promotes Gilthead Seabream larvae digestive capacity and modulates oxidative status. Animals. 11, 1667. https://doi.org/10.3390/ ani11061667
59. Xavier MJ, Engrola S, Conceicao LEC, Manchado M, Carballo C, Goncalves R, Colen R, Figueiredo V, Valente LMP. 2020. Dietary antioxidant supplementation promotes growth in Senegalese sole. Front. Physiol. 12 doi.org/10.3389/fphys.2020.580600.
60. Xu MY, Volkoff H. 2009. Molecular characterization of ghrelin and gastrin releasing peptide in Atlantic cod (*Gadus morhua*): cloning, localization, developmental profile and role in food intake regulation. Gen. Comp. Endocrinol. 160, 250-258.
61. Yang S, Liu Z, Yan Z, Zhao Z, Zhang C, Gong Q, Du X, Wu J, Feng Y, Du J, Huang X,. 2021. Improvement of skeletal muscle growth y GH/IGF growth axis contributes to growth performance in commercial fleshy sturgeon. Aqua. 543, 736929.
62. Zhao N, Liao M, Wu J, Chu K. 2017. Curcumin suppresses Notch-1 signalling: improvements in fatty liver and insulin resistance in rats. Mol. Med. Rep. 17, 819-826.
63. Zhao TJ. 2010. Ghrelin O-acyl transferase (GOAT) is essential for growth hormone mediated survival of calorie restricted mice. Proc. Natl. Acad. Sci. 107, 7467-7472.
64. Zhou Y, Liang XF, Yuan X, Li J, He Y, Fang L, Guo X, Liu L, Li B, Shen D. 2013. Neuropeptide Y stimulates food intake and regulates metabolism in grass carp, *Ctenopharyngodon idellus*. Aqua. 380, 52-61.

Tables

Tables 1 to 3 are available in the Supplementary Files section

Figures

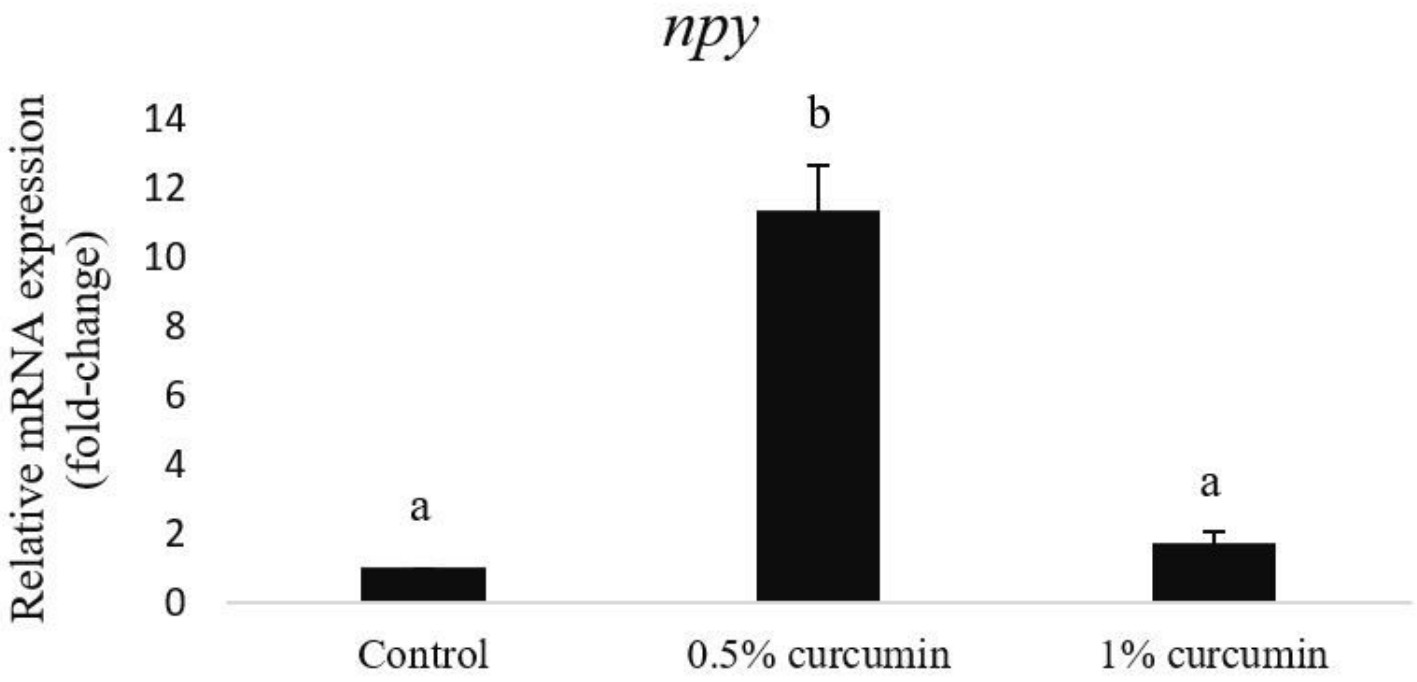

Figure 1

Effects of dietary curcumin for 100 days on the mRNA abundance of NPY in brain.

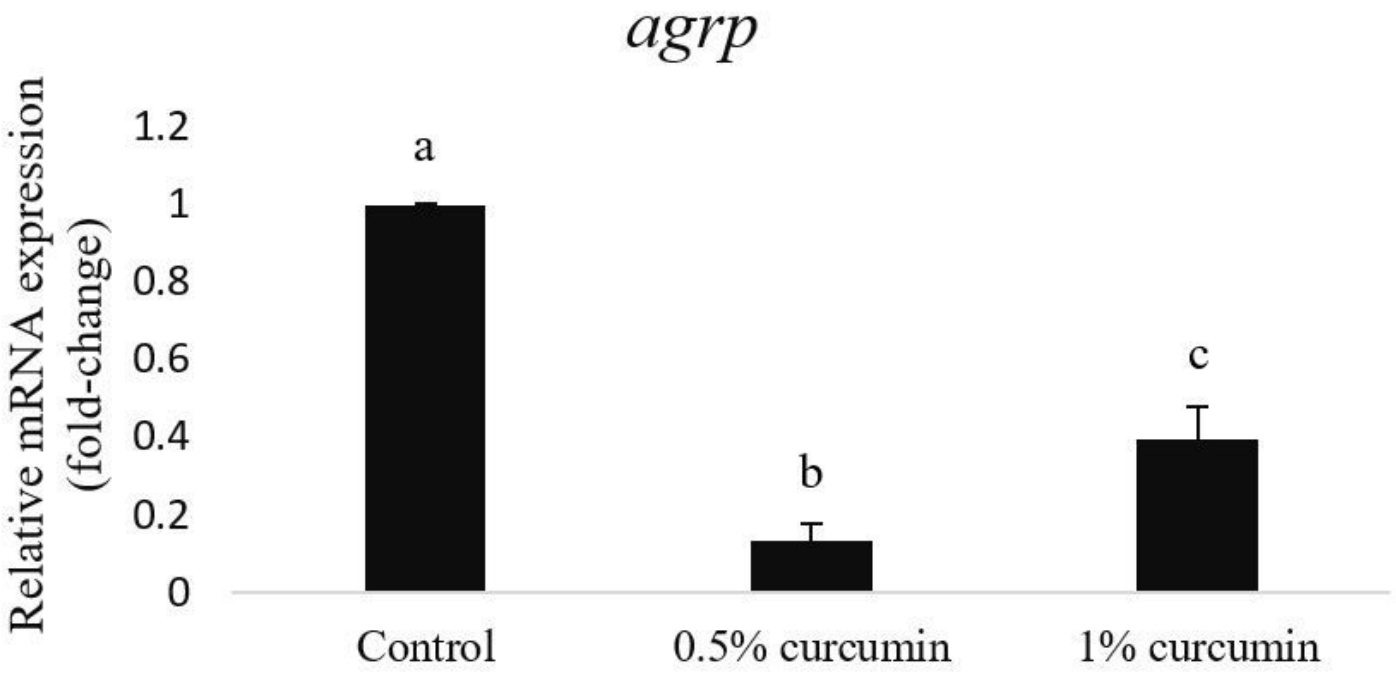

Figure 2

Effects of dietary curcumin for 100 days on the mRNA abundance of AgRP in brain.

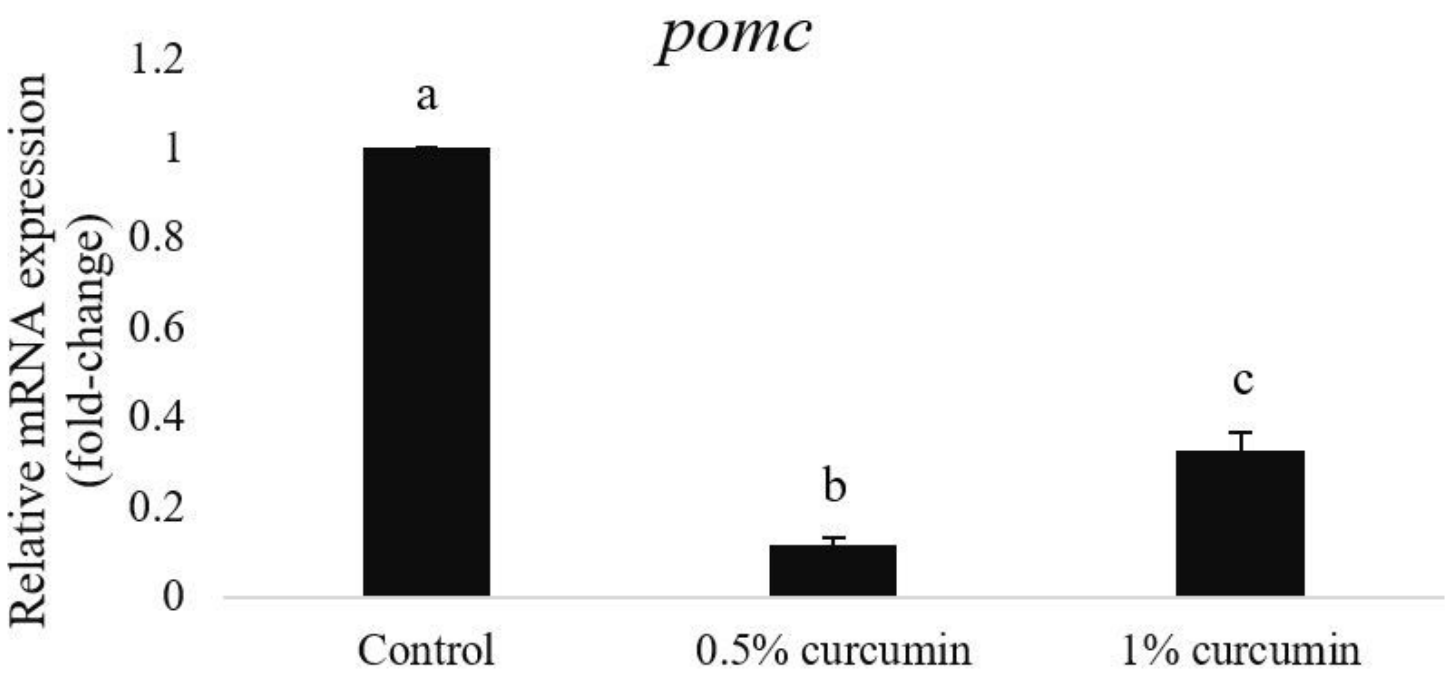

Figure 3

Effects of dietary curcumin for 100 days on the mRNA abundance of POMC in brain.

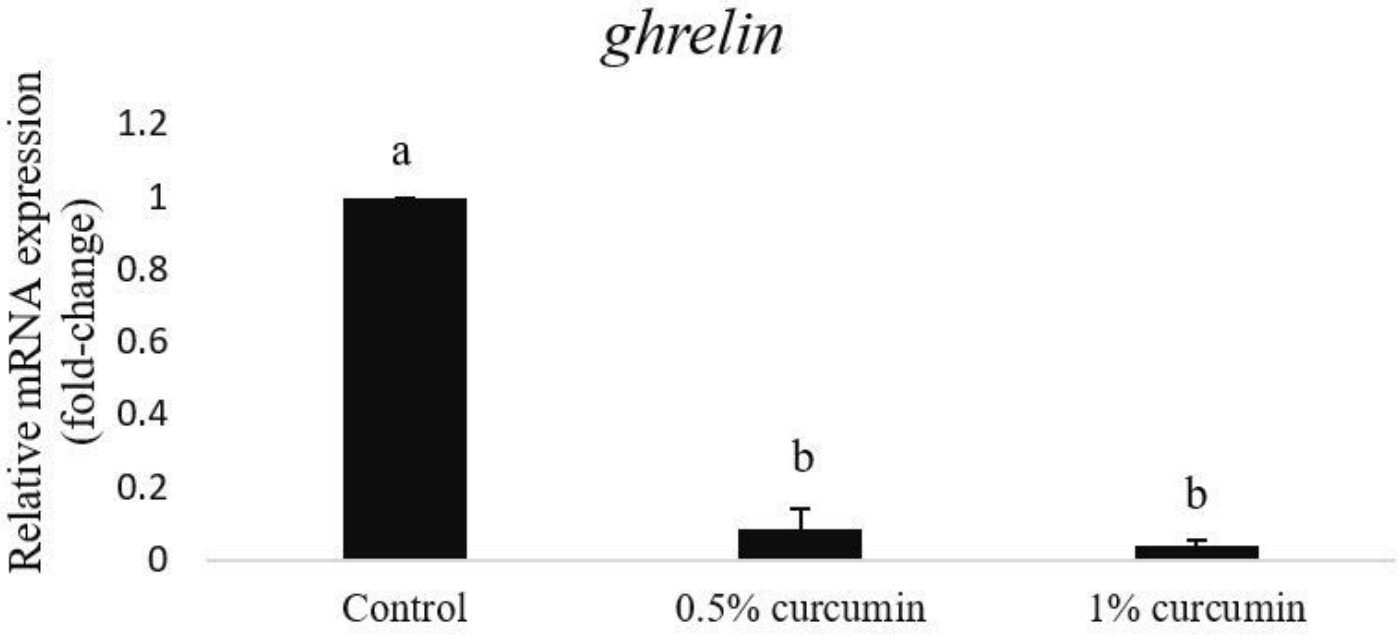

Figure 4

Effects of dietary curcumin for 100 days on the mRNA abundance of ghrelin in brain.

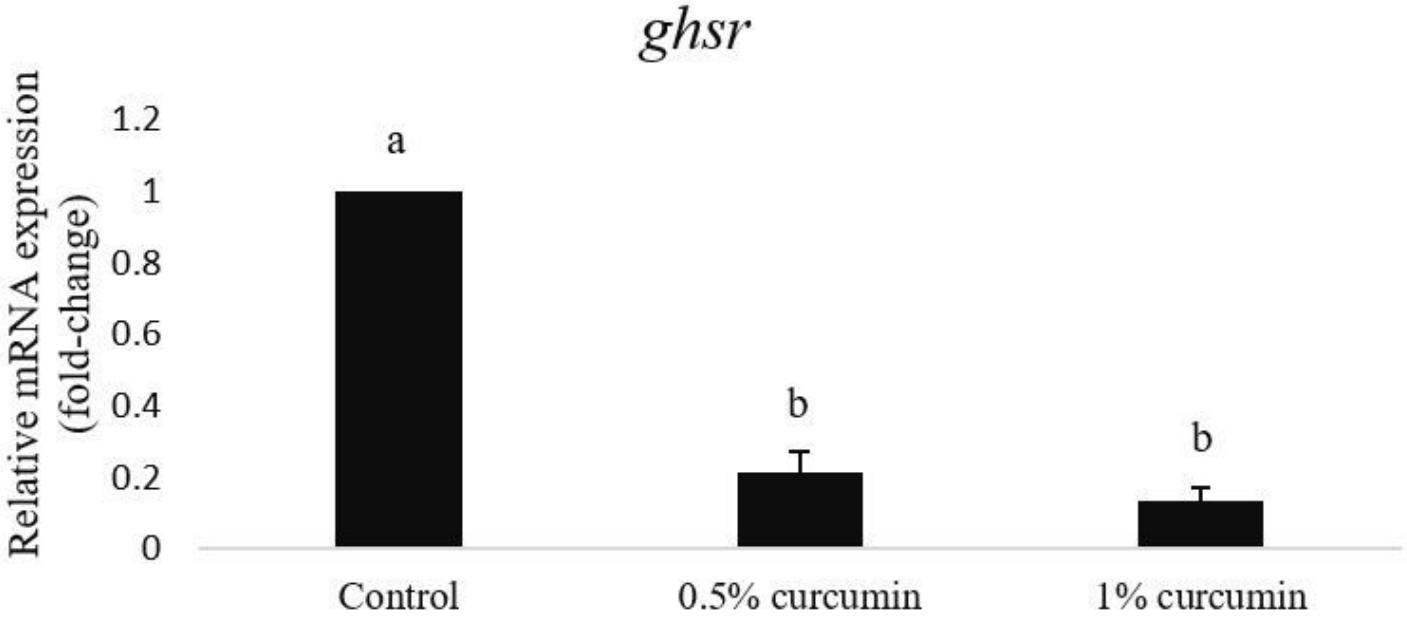

Figure 5

Effects of dietary curcumin for 100 days on the mRNA abundance of GHSR in brain.

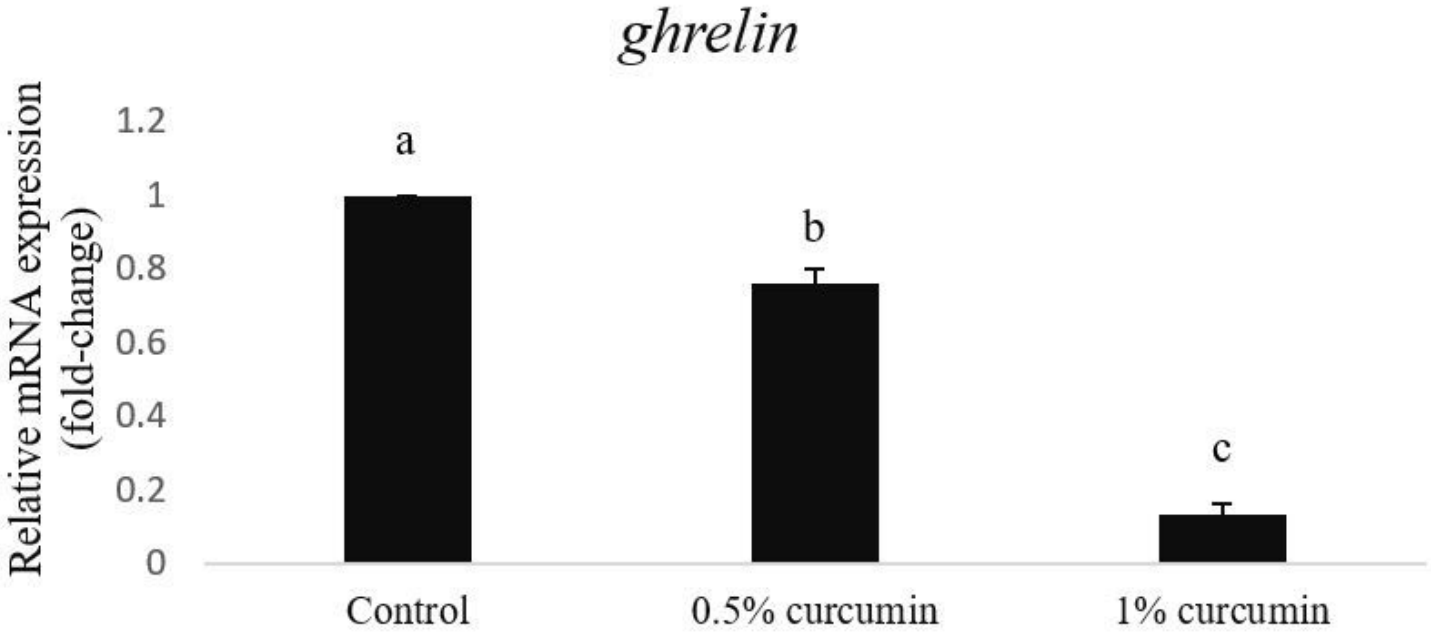

Figure 6

Effects of dietary curcumin for 100 days on the mRNA abundance of ghrelin in stomach.

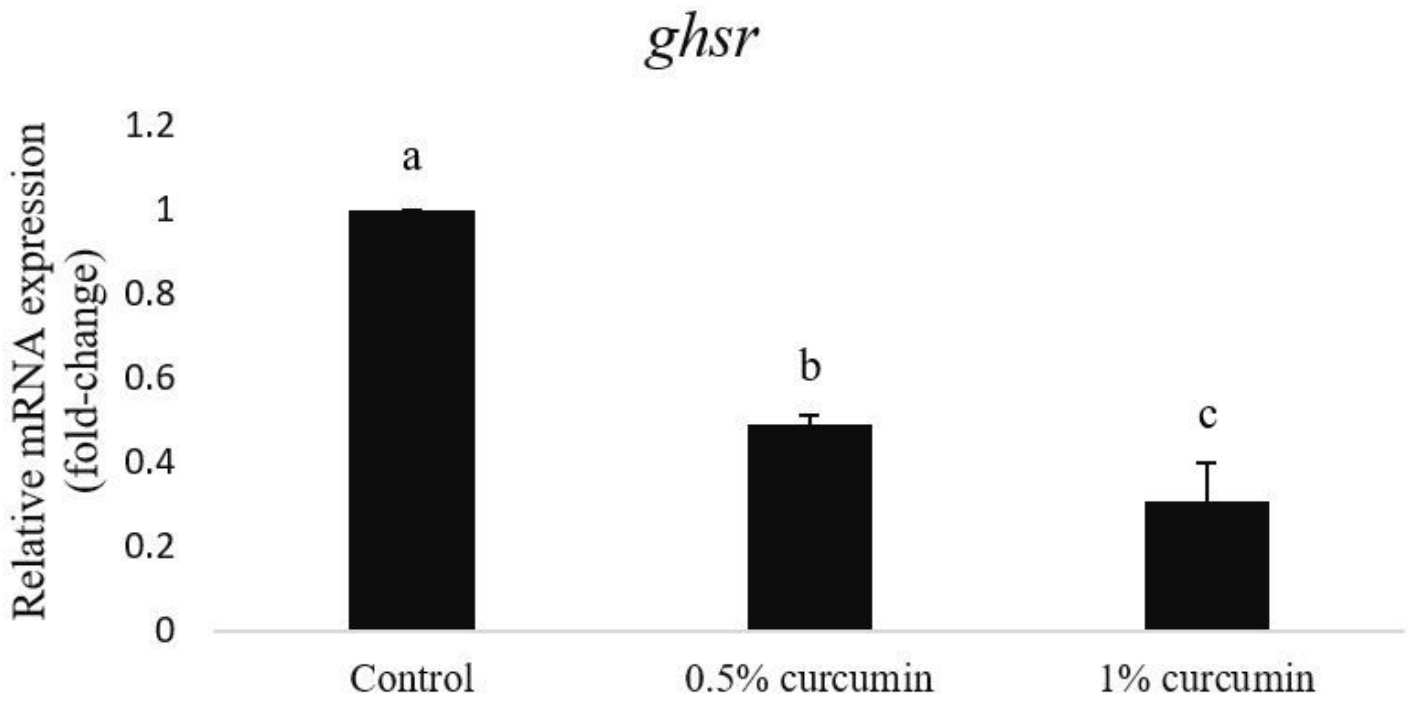

Figure 7

Effects of dietary curcumin for 100 days on the mRNA abundance of GHSR in stomach.

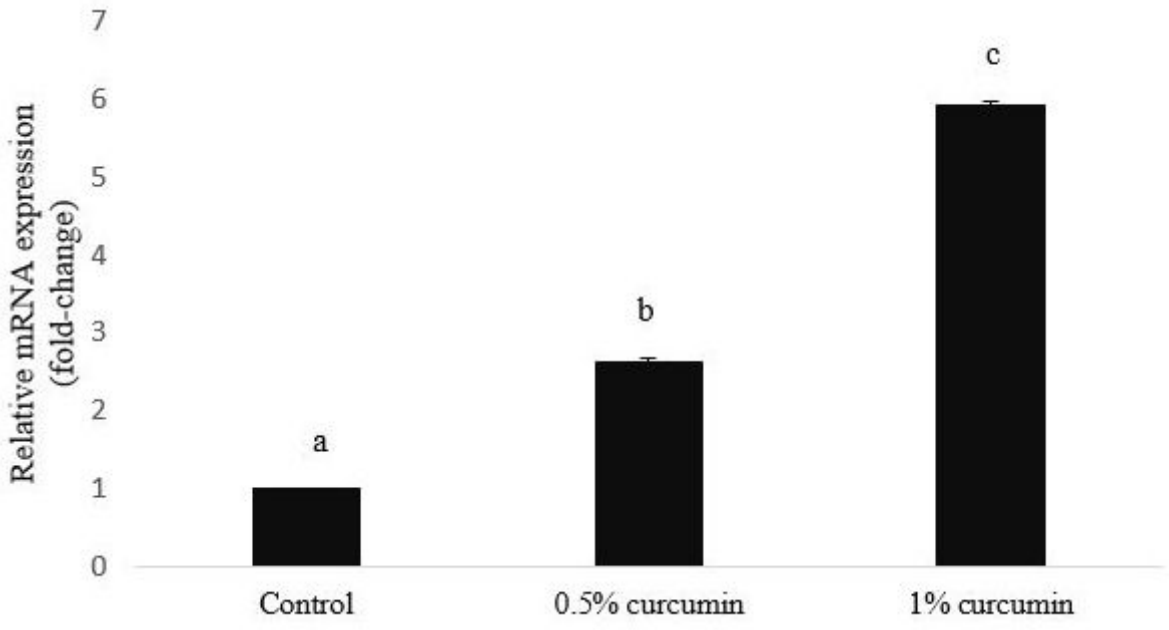

Figure 8

Effects of dietary curcumin for 100 days on the mRNA abundance of GHR in muscle.

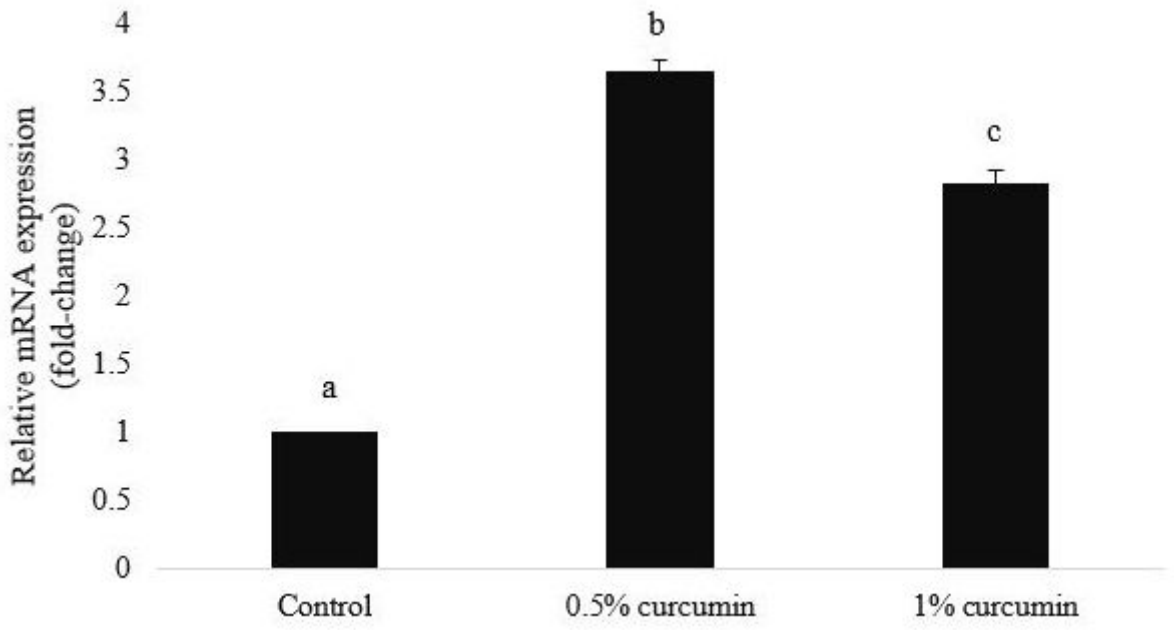

Figure 9

Effects of dietary curcumin for 100 days on the mRNA abundance of IGF1 in muscle.

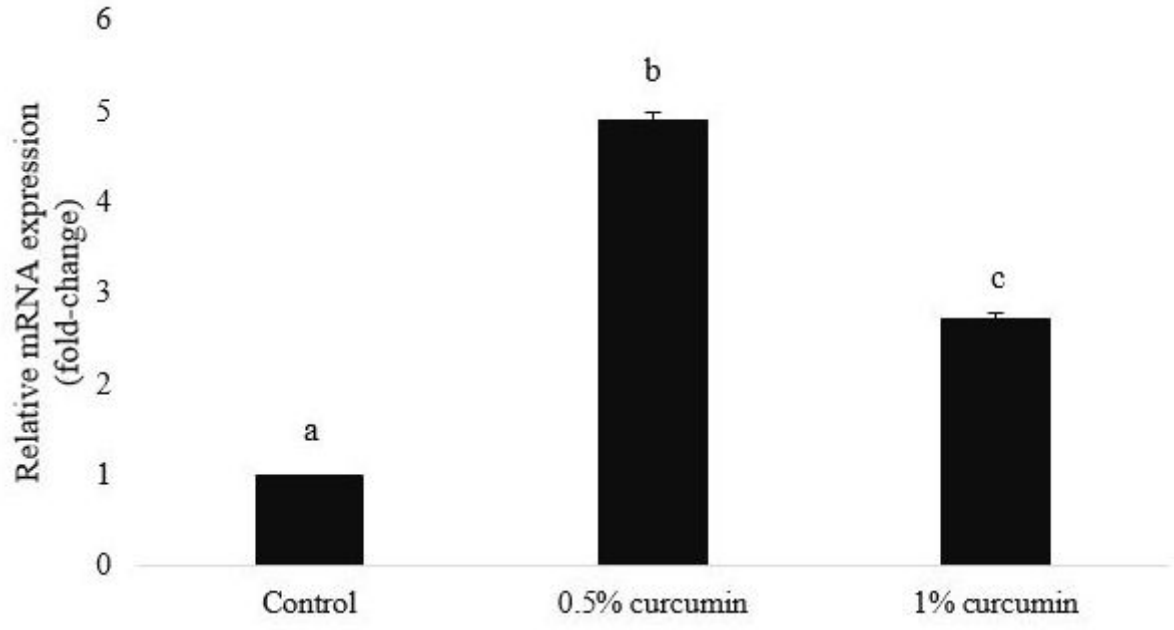

Figure 10

Effects of dietary curcumin for 100 days on the mRNA abundance of IGF2 in muscle.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1to3.docx