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Quercetin in the diet of silver catfish: Effects on antioxidant status, blood parameters and pituitary hormone expression

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ABSTRACT

We analysed the effects of quercetin-containing diet on blood parameters, antioxidant status and pituitary hormone expression in silver catfish. Diets containing three concentrations of quercetin (0, 0.15 and 0.30%) were provided to fish once a day. The results indicated that quercetin did not promote any significant change on the haematological and biochemical parameters measured. Fish that received the diet with quercetin presented decreased lipid peroxidation (LPO) (measured by lipid hydroperoxides and thiobarbituric acid reactive substances) in all tissues evaluated. On the other hand, the activity of antioxidant enzymes such as superoxide dismutase, catalase, glutathione peroxidase and glutathione S-transferase were higher in tissues of fish fed with diets containing quercetin. Additionally, the content of non-protein thiols, total reactive antioxidant potential and ascorbic acid were also higher in tissues of quercetin fed fish. Finally, there was no changes regarding cortisol levels and the expression of growth hormone, prolactin and somatolactin in fish fed with quercetin when compared with the control. Our results suggests that supplementation of silver catfish diet with quercetin is beneficial since it reduced the LPO and increased antioxidant capacity in vital tissues of fish without having any impact on haematological and biochemical parameters, and on pituitary hormone gene expression.

Statement of relevance: We propose quercetin as supplement in fish diets.

1. Introduction

The incorporation of food additives in the diet of fish aims to enhance performance, immunity and quality of fillet, since fish are susceptible to constant stress factors due intensive fish-farming practices that often cause poor health. In general, stressors cause an endocrine response in fish with activation of the hypothalamic–pituitary–interrenal axis, characterised by hypersecretion of catecholamines (epinephrine and norepinephrine) and cortisol. These hormones induce a number of secondary effects including rapid mobilisation of energy reserves (Tort, 2011). Furthermore, the fish pituitary hormones, such as growth hormone, prolactin and somatolactin are involved in stress responses and play a key role in regulating homeostasis of several physiological processes (Kaneko, 1996). Different stressors, such as salinity, confinement and food-deprivation, increased the expression of growth hormone, prolactin and somatolactin in gilthead sea bream (*Sparus aurata*) (Mancera et al., 2002; Laiz-Carrión et al., 2009). Aspects such as water quality, culture density, feeding, nutritional conditions, and handling procedures directly influence these hormonal pathways (Suárez et al., 2015). This stress response might lead to physiological unbalance leading to excessive formation of reactive oxygen species (ROS). Increased ROS levels can potentially damage cellular components, promoting lipid peroxidation (LPO), enzyme inactivation and oxidative DNA damage (Cho and Lee, 2012; Saleh et al., 2015).

The improvement of fish-farming conditions would benefit the welfare of fish as well as increase producers' profit. The use of natural antioxidants in fish diet could reduce production costs and offer an environmentally friendly alternative to synthetic compounds (Zheng et al., 2009; Shin et al., 2010; Awad et al., 2013; Saccol et al., 2013; Zhai and Liu, 2013; Pês et al., 2015).

Quercetin is a flavonol found widely in fruits, vegetables and nuts. In nature it exists primarily as quercetin glycoside and consists of quercetin aglycone conjugated to sugar moieties such as glucose or rutinose (Guo

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and Bruno, 2015). Kawabata et al. (2009) reported that oral administration of quercetin to rats affected the hypothalamic–pituitary–adrenal axis. These effects were associated to reduced stress as a consequence of decreased cortisol levels. This flavonoid is also described as an exceptional scavenger of free radicals such as peroxynitrite and hydroxyl radical (Bors et al., 1994; D'Andrea, 2015). This evidence supports the study of quercetin as a potential natural antioxidant to be incorporated in fish diet.

Silver catfish (*Rhamdia quelen*) has potential for aquaculture in southern Brazil due to its elevated growth rate, good carcass yield and easily controlled reproduction in subtropical climate (Baldisserotto, 2009). The current study aims to examine the effect of quercetin on blood parameters, oxidative biomarkers and pituitary hormone expression in *R. quelen*.

2. Material and methods

2.1. Fish

Silver catfish (215.19 ± 1.04 g, 23.65 ± 0.33 cm) were obtained from a fish culture sector of Federal University of Santa Maria (UFSM), Rio Grande do Sul (RS), Brazil. Experiments were conducted in a recirculating aquaculture system in the Fish Physiology Laboratory at UFSM. Animals were randomly distributed in nine plastic boxes (40 L), four fish per box, and acclimated to the laboratory conditions for two weeks. Water parameters were checked daily (temperature, total ammonia and dissolved oxygen) or weekly (alkalinity, total hardness and pH). The experimental protocol was approved by the Committee on Animal Experimentation of UFSM under registration no. 077/2013.

2.2. Water parameters

Temperature and dissolved oxygen levels were measured with a YSI oxygen metre (Model Y5512; YSI Inc., Yellow Springs, OH, USA). Temperature was maintained at 23.01 ± 0.03 °C and dissolved oxygen levels at 6.81 ± 0.12 mg L^{-1}. pH was verified with a DMPH-2 pH metre and the mean value was 7.32 ± 0.05 (Digimed, São Paulo, SP, Brazil). Nesslerization was used to verify the total ammonia nitrogen levels according to the method of Eaton et al. (2005). Non-ionised ammonia levels were calculated according to the method of Colt (2002). Water hardness was analysed by the EDTA titrimetric method. Alkalinity was determined according to the method of Boyd and Tucker (1992). The mean values of these parameters were as follows: total ammonia (2.51 ± 0.5 mg L^{-1}), non-ionised ammonia (0.09 ± 0.005 mg L^{-1}), hardness (20.7 ± 1.0 mg L^{-1} $CaCO_3$) and alkalinity (24.1 ± 1.3 mg L^{-1} $CaCO_3$).

2.3. Quercetin and reagents

Quercetin ($C_{15}H_{10}O_7$) was obtained from Opção Fênix Petrochemicals Distributor Ltd. (São Paulo, SP, Brazil). All of the other reagent-grade chemicals were obtained from Sigma (St. Louis, Missouri, USA).

2.4. Diets and experimental design

Three diets were formulated based on the study of Pês et al. (2015). The diet consisted of 30% soybean meal, 35% meat and bone meal, 12% rice bran, 15% corn, 3% canola oil, 1% salt, 3% vitamins and minerals (premix) and 1% phosphate dicalcium. The different concentrations of quercetin (0, 0.15 and 0.30%) were added to the mixture with rice bran. Water was added to the diets, and a drying process was performed in a forced air circulation oven for 24 h (45 °C). Such concentrations were chosen based on previous studies using dietary quercetin supplementation for other fish species (Shin et al., 2010; Awad et al., 2013). Fish received the experimental diets until apparent satiation once a day (9 a.m.) for 21 days.

The experimental design included in three groups (in triplicate), one for each quercetin. After 21 days, blood samples were collected from the caudal vein with heparinized sterile syringes and biochemical analysis was performed. Blood was sampled in less than 1 min and no anaesthetic was used for the groups, since previous studies have shown that the use of anaesthetic may affect the stress response (Small, 2003; Velisek et al., 2011; Gressler et al., 2014). Fish were euthanised by sectioning the spinal cord and pituitary, brain, gill, liver, kidney and muscle were removed and immediately frozen in liquid nitrogen. Tissues were stored at −80 °C for further analysis.

2.5. Total phenolic compounds

Total phenolic compounds were determined in the diets according to the Folin-Ciocalteau procedure as described in the study by Finamor et al. (2012). Gallic acid was used as a standard, and the results were expressed as gallic acid equivalents (mg GAE) 100 g of grain (dry weight). The samples analysed showed different concentrations of total phenolic compounds, which were higher in diets with 0.15% (125.97 mg GAE 100 g $diet^{-1}$) and 0.30% (115.29 mg GAE 100 g $diet^{-1}$) of quercetin, than in control (75.11 mg GAE 100 g $diet^{-1}$) ($P < 0.05$).

2.6. Haematological and biochemical analysis

Blood was utilised for different analysis. An aliquot of blood was used to determination of haematocrit (HCT) using microhaematocrit capillary tubes. The haemoglobin concentration (HB) was obtained using the Drabkin reagent (Kamper and Zijlstra, 1964), read spectrophotometrically at 540 nm and expressed as mmol L^{-1} blood. The mean cell haemoglobin concentration (MCHC) was calculated using the equation [Hb] * 100 / Hct and expressed as mmol L^{-1}.

Another aliquot of whole blood was subsequently transferred to microcentrifuge tubes and centrifuged at 3000 × *g* for 10 min (Centrifuge 5804 R) to obtain the plasma for biochemical analysis. Plasma cortisol levels were quantified using an enzyme-linked immunosorbent assay (ELISA) kit (Diagnostics Biochem Canada Inc., Canada), similarly to the study of Gressler et al. (2015). The samples were measured in duplicate, and the absorbance was determined in a spectrophotometer at 450 nm. The inter- and intra-assay variation coefficients were 5.15 ± 0.53 and 4.13 ± 0.67%, respectively. The results are presented as nmol L^{-1}. The levels of glucose (GLU), lactate dehydrogenase (LDH), triglycerides (TRI), cholesterol (CHO), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and urea (URE) in plasma were determined using commercial kits (Labtest, Minas Gerais, Brazil) and expressed as mmol L^{-1} and LDH as μkat L^{-1}.

2.7. Prooxidants and antioxidants analyses in tissues

For the measurement of oxidative stress biomarkers, each tissue was homogenised in 154 mmol L^{-1} KCl containing 1 mmol L^{-1} phenylmethylsulfonyl fluoride and centrifuged at 700 *g* for 10 min

Table 1
Primers design for amplification of β-actin, growth hormone, prolactin and somatolactin genes based on the sequences of these genes according with Baldisserotto et al. (2014).

Gene		Sequence
β-Actin	Forward	5′-CGA ATG CCA GGG TAC ATG GT-3′
	Reverse	5′-CCA CCT TCA ACT CCA TCA TTGA A-3′
Growth hormone	Forward	5′-TTG ACA GTC TTG GTG CTG CTT T-3′
	Reverse	5′-GAG CGA CTG CGT TGT TGA AG-3′
Prolactin	Forward	5′-ACC AGA GAC AGG AGC TCG TTC T-3′
	Reverse	5′-AGC TCA TGA GAC CGT CCA TGT-3′
Somatolactin	Forward	5′-CGA GGC CAG GAC TTT GTT TG-3′
	Reverse	5′-GAC GCG CAC AAG GTT TGA T-3′