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# Phytic acid antioxidant effects on *Rhamdia quelen* and *Cyprinus carpio* as potential farming supplementation against water pollution

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**ABSTRACT.** Fish and fishing products are among the most traded food types worldwide. Thus, the use of economically viable and sustainable diet supplementation alternatives to ensure animal health improvement is increasingly requested. Furthermore, the adoption of agricultural waste is a sustainable activity that helps reducing environmental pollution. The aim of the present study is to assess the antioxidant effect of a diet based on different rice bran phytic acid (PA) concentrations, namely: 0.5, 1.0, 1.5 and 2.0% by using oxidative stress and detoxification biomarkers in fish species *Cyprinus carpio* and *Rhamdia quelen*. The activity of glutathione S-transferase (GST) and hepatic catalase (CAT) in *C. carpio* decreased at 2.0% PA. Lipid peroxidation (LPO) increased in the liver at 2.0% PA and the carbonylated protein (CP) content decreased at all tested concentrations. The activity of GST increased in *R. quelen* at 2.0% PA, whereas CAT activity decreased at 0.5 and 1.5% PA. According to the current study, phytic acid might bring benefits to fish at concentrations up to 1.5% PA. In addition, adding this antioxidant to the feeding of fish bred in ponds can even lead to more significant effects.

**Keywords:** Aquatic pollution; antioxidant capacity; biomarkers; fish farming; rice bran.

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## Introduction

Fish and fishing products are among the most traded food types worldwide (Organização para a Cooperação e Desenvolvimento Económico/Organização das Nações Unidas para a Alimentação e a Agricultura [OECD/FAO], 2019) due to their high nutritional value, high protein content and essential micronutrients. Overall, the fishing and aquaculture sectors recorded expansion in production, trading and consumption in 2018, when it reached historical peaks. Estimates show that the total amount of bred fish will globally grow in the next years ([OECD/FAO], 2019).

Freshwater fish farming is a well outspread activity in Brazil (Barros & Martins, 2012), since the country presents favorable features for fish farming such as the wide territorial extension and varied climatic diversity. Thus, many fish species can be exploited by this economic activity (Fracalossi et al., 2004). Common carp (*Cyprinus carpio*) is one of the oldest freshwater species used for commercial production because it easily adapts to different climates; therefore, it has global relevance (Kong et al., 2016; Vandeputte, 2003). Silver catfish (*Rhamdia quelen*) is a neotropical fish native to South America; it is commonly bred in fish farming (Brito et al., 2017) and is largely accepted by the consumer market.

Several variables must be assessed in combination in order for fish farmers to achieve good fish yield. It is essential having a good nutritionally balanced diet, as well as ideal abiotic conditions, for fish breeding. The entry of chemical compounds from sources external to fishponds, for example, can become a stress factor for these confined aquatic organisms. Contamination by xenobiotics, such as pesticides and fertilizers used in agricultural activities, besides domestic sewage (of the most varied compositions) is among problems related to water resources (Pérez et al., 2018; Vieira et al., 2019; Storck et al., 2022, 2025). The negative effects of environmental pollutants on fish health are well known (Ghisi et al., 2014; Ribeiro et al., 2014).

Aquatic environment pollution compromises fish development due to adverse effects stemming from contact with xenobiotics. It is possible observing increase in the activity of detoxification enzymes such as glutathione S-transferase (GST) when this organism is exposed to a toxic compound. It happens to help eliminating this substance. Moreover, oxidative stress is one of the main effects caused by pollutants in fish, at cell level (Costa-Silva et al., 2015; Clasen et al., 2018), and it results from increase in reactive oxygen species (ROS) concentration due to imbalance between such compounds' production and elimination from this organism. Oxidative stress can damage cell components (proteins and lipids) and disturb the normal functioning of the metabolism (Lushchak, 2011). Catalase (CAT) is an enzymatic antioxidant agent that turns hydrogen peroxide ($H_2O_2$) into $O_2$ and water. This enzyme's activity assessment is often reported as oxidative stress biomarker (Das et al., 2017; Amaral et al., 2018). Organisms get endogenous antioxidants and exogenous antioxidants, among them vitamins A, C, and E, carotenoids, flavonoids (Peterson, 2001), selenium (Menezes et al., 2013; Monteiro et al., 2009) and phytic acid (Harbach et al., 2007; da Costa et al., 2021) through the ingested diet. Therefore, fish enzymatic and non-enzymatic antioxidant defense system, as well as detoxification enzymes, act to protect these organisms from the negative effects of aquatic contaminants like free radicals and ROS (Young & Woodside, 2001).

Phytic acid (PA) is among the potential supplementation substances capable of improving the health condition of bred fish (da Costa et al., 2021). Studies have proven that although this vegetal component is an anti-nutritional compound, it has beneficial effects, such as antioxidant potential (Graf & Eaton, 1990; Harbach et al., 2007). PA antioxidant properties are related to its binding affinity with iron (Graf & Eaton, 1990), whose chelation capacity renders this metal catalytically inactive, and it inhibits the induction of iron-mediated hydroxyl radicals (•OH) production. Thus, it changes iron redox potential ($Fe^{2+}$ to $Fe^{3+}$) and quickly removes $Fe^{2+}$ without any simultaneous •OH production. This process protects against cell damage because $Fe^{2+}$, alone, leads to oxy-radicals and lipid peroxidation production, whereas $Fe^{3+}$ is relatively inert (Graf et al., 1984, 1987). PA prevents $Fe^{3+}$ from participating in the Fenton reaction after it binds to Fe ions in solution; consequently, •OH formation due to $Fe^{2+}$ oxidation to $Fe^{3+}$ does not happen during $Fe^{2+}$ reaction with $H_2O_2$ or with peroxides (Bohn et al., 2008). However, little is known about the antioxidant effects of PA on fish.

The development of nutritionally balanced diets is necessary to improve bred species development given the expansion of the fish farming activity (Rodrigues et al., 2020), it also aims at mitigating all stressor effects affecting fish performance, as much as possible. In addition, economically viable alternatives for diet supplementation at low cost for producers are a target. Thus, rice bran is a rice processing industry byproduct presenting high PA concentration in its composition (Canan et al., 2011, 2012). The Brazilian Southern region stands out in the country's agricultural scenario for paddy rice cultivation (Rio Grande do Sul, 2020). Therefore, the waste generated by this industrial process let to the use of rice bran for other purposes. It became a sustainable and environmentally friendly alternative. The aim of the current study was to assess the antioxidant effect of a diet based on different concentrations of rice bran PA assessed through oxidative stress and detoxification biomarkers in *C. carpio* and *R. quelen*.

## Material and methods

### Diet composition

Phytic acid (PA) used in the diet of the fish was acquired from a food factory through processing rice bran (Santa Maria, RS, Brazil). Four diets were formulated with different percentages of PA (0.5%, 1.0%, 1.5%, and 2.0%), besides the feed used as the control (0.0% PA), based on the percentages used in previous studies (McClain & Gatlin, 1988; Usmani & Jafri, 2002). Diets were produced according to da Costa et al. (2021). The base diet composition was the same in all treatments, including the control, with 37% of crude protein, 3250 kcal $kg^{-1}$ of digestible energy, and 12% of lipids (Salhi et al., 2004).

### Animals

*Rhamdia quelen* (4.8 ± 0.04 cm and 5.0 ± 0.05g) and *Cyprinus carpio* (9.0 ± 0.07 cm and 8.0 ± 0.08g) were obtained from a fish farm (Santa Maria, RS, Brazil) and acclimated in 250 L polyethylene boxes, with clean water, aeration, a filtration system, and constant temperature for ten days. Water quality variables such as ammonia, nitrite, and pH were analyzed periodically. In this phase, the animals were fed with commercial feed twice a day, at 9:00 AM and 5:00 PM. This study was approved by the Ethics Committee of the *Universidade Federal de Santa Maria* (protocol No. 8087140916).

### Experimental design

Separated by species, the fish were distributed randomly in 50 L tanks with clean water and with n = 9 in each. Both species were fed for fifty days with the feeds containing different percentages of PA. Treatment 1 was composed of the control group, without the addition of PA in the diet composition (0.0% PA), while the diets in the other treatments were provided with 0.5, 1.0, 1.5, and 2.0% of PA, denominated 0.5% PA, 1.0% PA, 1.5% PA, and 2.0% PA, respectively, all in triplicate. After the experimental period, the animals were anesthetized with eugenol and euthanized by spinal cord sectioning. The hepatic tissues of *C. carpio* and *R. quelen* were collected and stored in liquid nitrogen for posterior biochemical analyses.

### Biochemical analyses

The fish livers were homogenized with a 50 mM Tris-HCl buffer, pH 7.5, centrifuged at 1,400 g for 10 min, and the supernatant was used for the biochemical analyses. The biochemical analyses were modified for microplate, except for catalase (CAT). Glutathione S-transferase (GST) activity was measured following Habig et al. (1974) using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate and expressed as µmol GS-DNB min-1 $mg^{-1}$ of protein. CAT activity was determined as per Aebi (1984) through the principle of the decrease in absorbance of hydrogen peroxide, metabolized by catalase, expressed as µmol $min^{-1}$ $mg^{-1}$ of protein. Regarding the oxidative damage, lipid peroxidation (LPO) was assessed by the quantification of one of its final products, malondialdehyde (MDA), through substances reactive to thiobarbituric acid (TBARS), as per Draper & Hadley (1990) and expressed in nmol MDA $mg^{-1}$ of protein. Carbonyl protein (CP) content was analyzed according to Yan et al. (1995) and expressed in nmol carbonyl $mg^{-1}$ of protein. The determination of the protein was carried out following Bradford (1976).

### Statistical analysis

The data were tested regarding homogeneity by the Shapiro-Wilk test. The comparison among treatments was carried out using a one-way analysis of variance (ANOVA), followed by Tukey's test, to verify the difference in the biochemical responses related to the diets. The results were expressed as mean ± standard deviation, and the significance level considered was $p < 0.05$.

## Results

### Cyprinus carpio

The activity of glutathione S-transferase (GST) and hepatic catalase (CAT) in *C. carpio* decreased at the concentration of 2.0% PA in comparison to the control (Figures 1A and 1B). The other treatments did not present significant differences from the control. With respect to oxidative damage, there was increase in lipid peroxidation (LPO) level in the liver at 2.0% PA (Figure 1C), whereas the carbonylated proteins (CP) content significantly decreased at all concentrations in comparison to the control (Figure 1D).

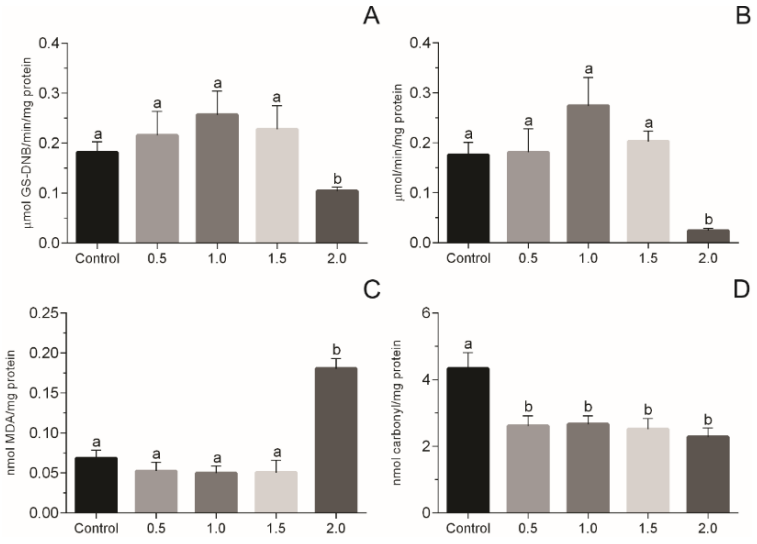


**Figure 1**. A: Glutathione S-transferase activity; B: Catalase activity; C: Lipid peroxidation level; and D: Carbonylated protein content in the liver of *Cyprinus carpio* fed for fifty days with diets based on different phytic acid concentrations (0.5%, 1.0%, 1.5% and 2.0%). Different letters correspond to significant difference among the groups ($P < 0.05$).

### Rhamdia quelen

The activity of GST in *R. quelen* increased at 2.0% PA in comparison to the control (Figure 2A), whereas the CAT activity significantly decreased at 0.5% and 1.5% PA in comparison to the control (Figure 2B). LPO level in the liver significantly increased at 2.0% PA in comparison to the control (Figure 2C). There was no difference in CP content among all groups (Figure 2D).

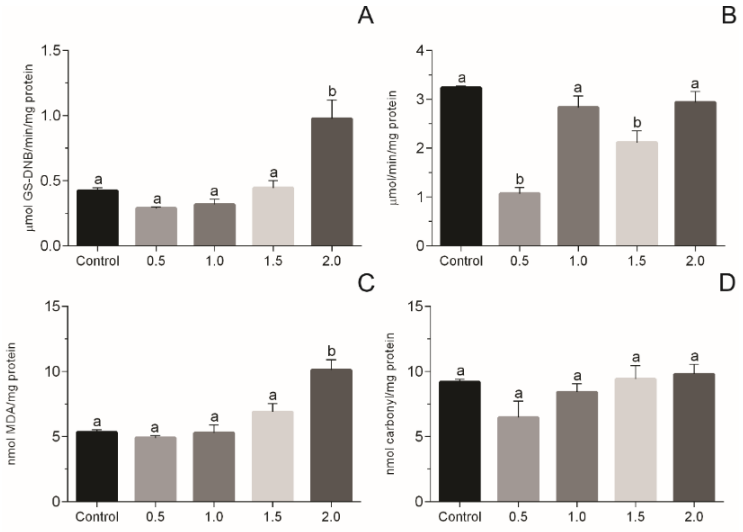


**Figure 2**. A: Glutathione S-transferase activity; B: Catalase activity; C: Lipid peroxidation level; and D: Carbonylated protein content in the liver of *Rhamdia quelen* fed for fifty days with diets based on different phytic acid concentrations (0.5%, 1.0%, 1.5% and 2.0%). Different letters correspond to significant difference among the groups ($P < 0.05$).

## Discussion

Currently, the livestock sector seeks economically and environmentally viable alternatives for animal farming supplementation good enough to improve bred animals' health condition. Rice bran is a rice processing byproduct that could be used to enrich the diet of confined animals potentially exposed to contaminants such as fish, mainly due to the presence of PA in it. This compound could reduce the risk of increased reactive oxygen species (ROS) production or improve antioxidant defenses (da Costa et al., 2021) in fish bred in dams located in agricultural and residential areas given its chelating action upon binding to metals like iron.

According to the present results, the enzymatic activity of GST and CAT increased after the ingestion of diets based on 0.5, 1.0 and 1.5% PA in *C. carpio*; however, there was no significant difference in comparison to the control. On the other hand, the diet based on 2.0% PA showed reduced activity of these enzymes, as well as significant increase in LPO level, i.e., increase in lipid peroxidation in the liver. CAT activity significantly decreased in the groups supplemented with 0.5 and 1.5% PA in *R. quelen*. GST activity increase took place along with increase in LPO levels after the diet based on 2.0% PA, in this same species. Accordingly, the addition of 2.0% PA in the diet of both carp and silver catfish led to negative effects, especially due to increase in hepatic LPO levels. LPO is a biochemical disturbance that emerges when the organism is under oxidative stress, since it can damage lipids in cells (Van der Oost et al., 2003).

Phytase is the enzyme responsible for degrading PA and, consequently, for making it bioavailable. This enzyme is found in the intestines of fish such as grass carp (*Ctenopharyngodon idella*) (Huang et al., 2009) and common carp (*C. carpio*) at small amounts; and in tilapia (*Oreochromis niloticus*) (Ellestad et al., 2002) in larger amounts. Furthermore, it is highly dependent on intestinal pH (Baruah et al., 2004). There may be differences in these organisms' responses after PA is introduced in fish diet due to their distinct dietary habits, anatomy and physiology (Ferreira & Flora, 2017). Therefore, the supply of ideal PA concentrations in fish diet is extremely important, since this compound accounts for low digestibility. Yet, increase in water phosphorous compounds can end up causing environmental issues such as fishpond eutrophication (Baruah et al., 2004).

PA effects on fish are also related to the concentration of this compound in fish diet due to its non-specific chelating action. Phytate has chelating action in combination to cations such as potassium, magnesium, calcium, zinc, iron and copper because of the high density of negatively charged phosphate groups, besides

the formation of complexes with proteins and amino acids that reduce these nutrients' digestibility (Baruah et al., 2004; Bohn et al., 2008; Kumar et al., 2011) and compromise the availability of essential minerals (Denstadli et al., 2006). This nutritional deficiency entails various adverse effects on organisms (Ogino & Yang, 1978; Helland et al., 2006; Danwitz & Schulz, 2020).

Fish species *C. carpio* and *R. quelen* present anatomical differences (they are agastric and gastric, respectively) assumingly related to different responses by the herein assessed biomarkers. Stomach pH is acid in most fish - it ranges from 2 to 4; however, food digestion happens in a more alkaline environment in gastric fish (Rotta, 2003). There are PA-degrading enzymes that work at high activity at acid pH values close to 5, whereas other enzymes get such an activity level in alkaline pH values close 8; however, most of these enzymes accomplish better activity in pH values ranging from 4 and 6 (Konietzny & Greiner, 2003). They are very sensitive to pH variations; very acid or very alkaline environments can make them irreversibly inactive (Scottá et al., 2014). Caps' intestinal pH ranges from 6 and 7 (Rotta, 2003) and these values comply with the optimal pH for PA degradation by phytases. This finding can be related to the carbonyl protein content decrease observed in *C. carpio* due to likely better PA absorption and use.

## Conclusion

Phytic acid (PA) addition to fish diet proved beneficial at concentrations up to 1.5%, mainly for reducing hepatic protein carbonylation in *Cyprinus carpio*. Furthermore, for economic and practical reasons, 0.5% PA is enough to supplement the *C. carpio* diet. The addition of the lowest PA concentration to the *Rhamdia quelen* diet changed the catalase activity; therefore, further studies are recommended to assess this change. Therefore, using rice bran PA becomes an economically viable alternative for *C. carpio* fish farming. Finally, the use of AF can partially mitigate the effects of oxidative stress induced by environmental contaminants in fish farming systems located in agricultural areas, although further studies are needed to confirm this application under direct exposure to pollutants.

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