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Iron bioavailability in tambaqui (*Colossoma macropomum*) desiccated gill and liver powder: Study in rats

Francisca das Chagas do Amaral Souza[1]*, Jaime Paiva Lopes Aguiar[1], Lucia Kiyoko Ozaki Yuyama[1#], Bruna Quara de Carvalho Santos[2], Eudevan Souza Gomes[2] and Risonilce Fernandes de Sousa[1]

[1]Instituto Nacional de Pesquisas da Amazônia - Coordenação Sociedade Saúde Ambiente- Laboratório de Alimentos e Nutrição - Avenida André Araújo, 2936, Aleixo CEP: 69060001- Manaus-AM, Brazil.
[2]PIBIC/CNPq/INPA Grantee - Avenida Rodrigo Otávio, 3000. Coroado CEP: 69077-000- Manaus-AM, Brazil.

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This study assessed iron bioavailability in rats from diets enriched with desiccated tambaqui gill and liver powders using the hemoglobin depletion-repletion method. Tambaqui (Colossoma macropomum) liver and gills were bought at Manaus. After processing the livers and gills were placed on trays and desiccated in a ventilated incubator at 60°C. The rats were given free access to chow and water. The moisture, protein, fat, and iron contents of the chow were determined three times. Iron bioavailability was measured by the hemoglobin depletion-repletion method. We used 24 anemic animals, which were randomly selected and distributed into three groups of eight animals each: 1) Control Group – anemic rats fed a casein-based diet (AIN 93); 2) Experimental Group – anemic rats fed AIN 93 and desiccated tambaqui liver; 2A) Experimental Group – anemic rats fed AIN 93 and desiccated tambaqui gill; and 3) Pair-feeding Group – anemic rats, distributed in random blocks according to hemoglobin concentration and weight, fed the average amount of AIN 93 consumed by the Control group. Gills are high in lipids. In addition to high lipid content, gill powder had considerable levels of protein and iron. The baseline hemoglobin of Groups 2 (liver) and 2A (gills) did not differ. After seven days, only Group 2 (liver) reached appropriate hemoglobin levels. In conclusion, iron in desiccated tambaqui liver powder is highly bioavailable. The iron in desiccated tambaqui gill powder is not as bioavailable as rats consuming this powder did not reach appropriate hemoglobin levels within the experimental period.

Key words: Bioavailability, iron, powder, liver, gills, tambaqui.

INTRODUCTION

Anemia is considered one of the great global public health problems, especially iron-deficiency anemia, responsible for 95% of all anemia cases (Torres et al., 1994). According to World Health Organization (OMS, 2001) estimates, approximately 50% of all children aged less than four years in developing countries are anemic, representing a severe human health problem as 55% of child deaths are related to malnutrition. Despite the commitment made by 170 countries, including Brazil, during the World Summit for Children held in 1992 in Rome, to prioritize the fight against iron-deficiency anemia, the problem persists. Fisberg et al. (2001) found

that 54% of children from 10 Brazilian capitals aged less than five years had iron deficiency. This situation led the National Sanitary Surveillance Agency to pass the National Policy for Brazilian Food and Nutrition, RDC n°. 344, on December 13, 2002. This policy aims to reduce iron-deficiency anemia by establishing the compulsory enrichment of wheat meal and corn flour with iron and folic acid (ANVISA, 2002). However, assessing iron bioavailability in ingredients is more important than fortifying foods with iron as high concentrations of iron do not necessarily translate to high utilization rates by the human body (Troari et al., 2005).

Approximately 217,000 tons of fish are caught annually in the Amazon (Val and Santos, 2009), and households in the region consume approximately 30.0 kg of fish per year, as opposed to 4.0 kg/year by Brazilians in general (IBGE, 2010). Therefore, fish is the main source of animal protein in the Amazon (Oetterer, 2006). Manaus is the main port of delivery for all this catch. The large amounts of byproducts obtained by processing can be used as raw material for feeds and add value to other products (Stori et al., 2002). Brazil discards approximately 50% of fish biomass (Pessatti, 2001). Using residues may solve the pollution problem caused by a substance that is difficult to discard and instead generate economic, social and environmental especially public health benefits. The present study chose tambaqui's (*Colossoma macropomum*) because it is the most cultivated species in the Amazon region (IBAMA, 2007), with an estimated production of 14,000 tons/year (Inoue and Boijink, 2011). Since large amounts of tambaqui liver and gills are discarded, the study hopes to find ways to use these outstanding iron sources, which may become very important in the fight against deficiencies, especially iron. Adequate iron intake depends not only on individual requirement but also on iron bioavailability in different foods. Although there is little information about the use of tambaqui liver and gills, we estimate that approximately three tons of these byproducts are discarded daily in Amazon Rivers. Although organs are degradable, large amounts pollute the environment and unbalance the ecosystem. Hence, the study aimed to process tambaqui gills and liver, to determine the nutritional constituents of their powders, and to measure their iron bioavailability in an experimental rat model.

MATERIALS AND METHODS

Tambaqui (*C. macropomum*) liver and gills were bought at Manaus' farmer's markets, placed in coolers, and transported to the Laboratory of Food and Nutrition – Laboratório de Alimentos e Nutrição/ Instituto Nacional de Pesquisas da Amazônia (LAN/INPA), Brazil. The organs were rinsed with tap water, boiled in a stainless steel pot for 10 min, dehydrated by placing even slices of approximately 1.0 cm of livers and gills on trays, and desiccated in a ventilated incubator at 60°C until the weight stabilized, indicating the moisture content. The samples were then ground by an electric grinder. The powder was stored in polyethylene packages until physical, chemical and microbiological analyses. In order to minimize metal contamination, especially iron, all glassware and utensils were rinsed with a 30% solution of nitric acid, rinsed with deionized water, and dried at least six times. Casein-based chows were prepared exactly as recommended by the American Institute of Nutrition – AIN-93G (Reeves et al., 1993). The animals had free access to food and water.

The moisture, protein, lipid (AOAC, 1995) and iron contents of the chow were determined three times by atomic absorption spectroscopy, as recommended by Institute Adolph Lutz (IAL, 2008), using the method provided by the Varian manual (VARIAN, 2000). The samples were digested in the microwave digester MARS (Xpress CEM Corporation, MD – 2591). The organic material was mineralized by concentrated nitric acid, cooled and diluted with deionized water. The iron contents of the diluted solutions were determined directly by atomic absorption spectroscopy (Spectra AA, model 220 FS, Varian, 2000), with specific lamps as instructed by the manufacturer. The analyses were controlled as instructed by the Varian Manual (Cornelis, 1992), using certified Peach leaves (NIST – SEM 1547) as reference.

The levels of *Salmonella*, total coliforms, fecal coliforms and *Escherichia coli* of the samples were determined by the International Commission on Microbiological Specifications for Foods (ICMSF) method, as required by RDC 12/01 (ANVISA, 2001) of the National Sanitary Surveillance Agency (ICMSF, 1983). This project was approved by the Animal Research Ethics Committee of the Federal University of Amazonas (UFAM) under protocol number 068/2012.

Iron bioavailability was determined by the hemoglobin depletion-repletion method. Wistar dams (*Rattus norvegicus* albinus, Rodentia, Mammalia) with six pups each (n=42) provided by the Animal Facility of the National Central Institute of Amazon Researches (INPA) were fed a casein-based chow without iron added to induce iron-deficiency anemia during the nursing period (21 days). After weaning, the pups were fed the same chow for another seven days. At the end of the depletion stage, blood was collected by sectioning the terminal portion of the tail to determine hemoglobin and select animals for the repletion stage. We used the cut-off points suggested by Margoles (1984): Anemia in rats is defined as Hb<7 g/dL and normal iron status as Hb>11 g/dL. For the 14-day iron repletion stage, anemic animals were randomly distributed into three groups with eight rats each: (1) Control group - anemic rats fed a casein-based diet (AIN 93); (2) experimental group - anemic rats fed AIN 93 and desiccated tambaqui liver powder as iron source; 2A) experimental group - anemic rats fed AIN 93 and desiccated tambaqui gill powder as iron source; and (3) Pair-feeding group - anemic rats fed the average amount of AIN 93 consumed by the control group, distributed into random blocks according to hemoglobin level and weight. During the iron depletion stage, the animals were housed in polypropylene boxes with

*Corresponding author. E-mail: francisca.souza@inpa.gov.br.

#In memoriam.

Table 1. Chemical composition of the chows used during iron depletion and repletion stages, Manaus (AM), 2013.

Ingredients	AIN 1993 G (%)*	AIN 1993 G (%)**	Liver powder (%)**	Gill powder (%)**
Casein	20.00	20.00	18.40	18.40
Sucrose	10.00	10.00	10.00	10.00
Soybean oil	7.00	7.00	7.00	7.00
Microcrystalline fiber	5.00	5.00	5.00	5.00
Saline	-	3.50	-	-
Saline without iron	3.50	-	3.50	3.50
Vitamin mix	1.00	1.00	1.00	1.00
L-cysteine	0.30	0.30	0.30	0.30
Choline bitartrate	0.25	0.25	0.25	0.25
Liver flour	-	-	4.00	-
Gill flour	-	-	-	4.00
Subtotal	`47.05	47.05	49.45	49.45
Corn starch	52.95	52.95	50.55	50.55
Total	100.00	100.00	100.00	100.00

*Chow during the depletion stage; **chow during the repletion stage; AIN 1993 G=American Institute of Nutrition.

Table 2. Proximate composition of desiccated tambaqui (*Colossoma macropomum*) liver and gill powders in 100 g of dry base. Manaus (AM), 2013.

Parameter	Tambaqui liver powder	*Raw beef liver	*Raw chicken liver	Tambaqui gill powder
Moisture (g)	2.77**	71.3	77.8	0.49**
Ash (g)	3.53	1.5	1.2	6.65
Proteins (g)	39.82	20.7	17.6	31.33
Lipids (g)	17.81	5.4	3.5	56.63
Iron (mg)	86.74	5.6	9.5	25.78

*Source: Brazilian Food Composition table (TACO). Food Study and Research Core (*Núcleo de Estudos e Pesquisa em Alimentação*, NEPA), UNICAMP. v.2. 4. ed. Campinas, 2011. 161 p. ** Residual moisture.

stainless steel lids, and in the repletion stage, they were housed in individual stainless steel cages under controlled humidity and temperature (~23°C) and 12 h light/dark cycles. The rats had free access to food and water. The chows used in the experimental period were prepared as recommended by Reeves et al. (1993) at Table 1, with 35 mg of iron/kg of chow. At the beginning and end of each repletion week, blood was collected by caudal vena cava puncture. Hemoglobin was determined by Hemo-Control microcuvettes and directly by the portable hemoglobinometer HemoCue®.

The results were submitted to analysis of variance (ANOVA). Statistical analyses were conducted by the software INSTAT version 3.0 and included the Tukey-Kramer comparisons at a significance level of 5% (Gomes, 1987).

RESULTS AND DISCUSSION

Salmonella sp. and total and fecal coliforms were not found in any of the samples. These findings confirm good manufacturing practices and proper hygienic conditions during processing, which is in agreement with Resolution RDC no. 12 passed on January 02, 2001 (Brasil, 2001). Table 2 shows the proximate composition of desiccated tambaqui gill powder. The gills have high lipid content, 56.63 g in 100 g of edible parts. For comparison, raw beef liver contains 5.4 g of lipids in 100 g of edible parts (TACO, 2011). Gill powder also contained high protein and iron contents. On the other hand, desiccated tambaqui liver powder has higher iron and protein contents than other foods (Table 2). The nutritiousness of this organ with respect to iron, lipids, and proteins is undeniable. At the end of the depletion period, the mean hemoglobin of the animals fed a low-iron chow was significantly lower, demonstrating that the methods were appropriate for the study objectives. These results are similar to laboratory results that used the same methods (Silva et al., 1998). During the repletion stage, the baseline hemoglobin of groups 2 and 2A (liver and gills) did not differ ($p<0.05$). On day seven, only the group consuming desiccated tambaqui liver powder reached normal hemoglobin levels ($p<0.05$). On day fourteen, all rats had higher hemoglobin levels ($p<0.05$), indicating the bioavailability of iron in desiccated tambaqui liver powder

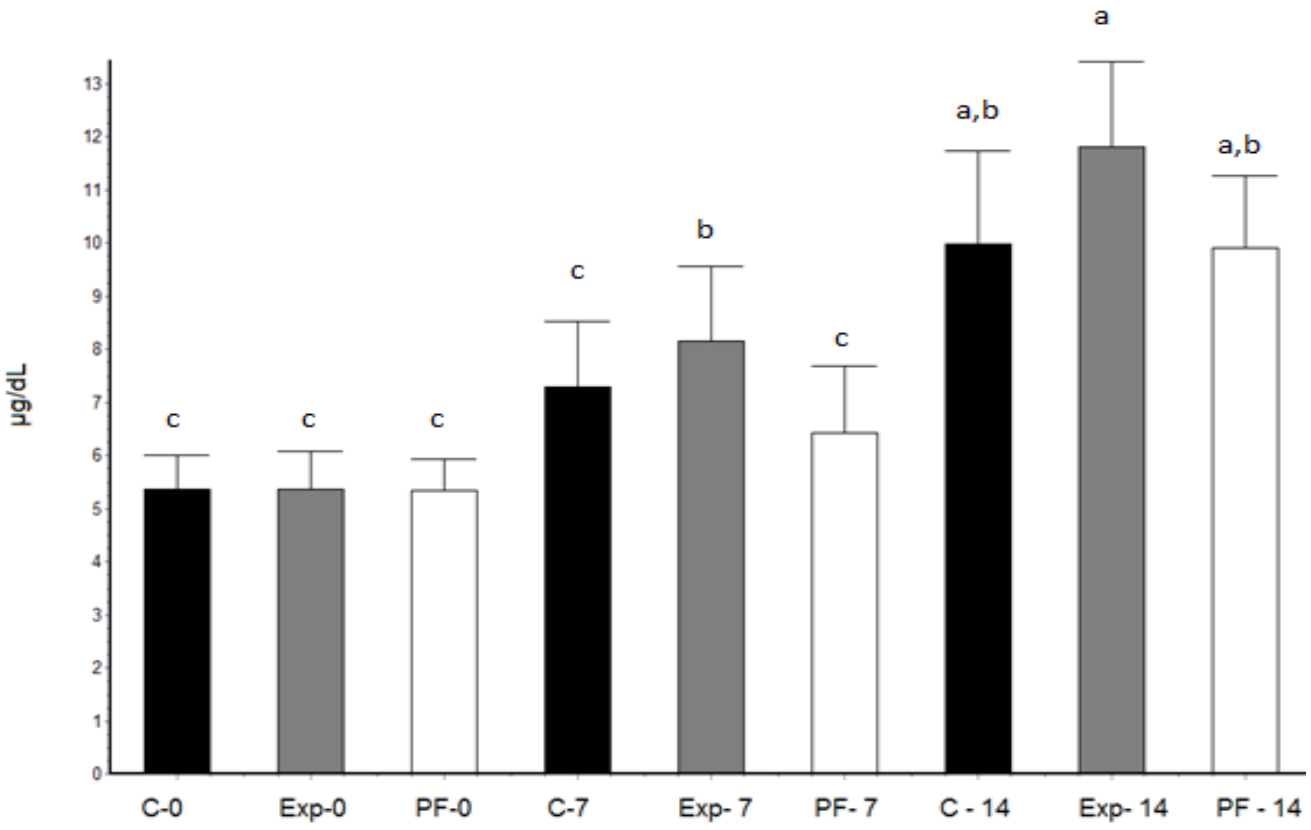

Figure 1. Hemoglobin levels (Hb) of animals in the control group (C), experimental group 2 (liver, Exp), and paired-feeding group (PF) at baseline (0), on day seven, and on day fourteen. Levels with the same letters are not significantly different according to the Tukey test ($p<0.05$).

(Figure 1). At baseline, the hemoglobin levels of the animals in the desiccated tambaqui gill powder group were not different from those of the other groups. However, on day seven, the hemoglobin levels of the groups differed significantly. On day fourteen, the hemoglobin levels of the experimental groups differed significantly from those of the control and pair-feeding groups, and the hemoglobin levels of all groups differed from those at baseline and on day seven (Figure 2). At baseline, all rats had similar weights. On days seven and fourteen, the rats in the group pair-feeding had gained significantly less weight than those in the other three groups ($p<0.05$) (Figure 3). The body weight of the rats that consumed desiccated tambaqui gill powder did not differ from that of the other groups at baseline and on day seven. However, on day fourteen, the control group differed from the other groups and from itself at baseline and on day seven (Figure 4).

Conclusion

In conclusion, iron in desiccated tambaqui gill powder is not as bioavailable, not helping anemic animals to recover normal hemoglobin levels. Thus, desiccated tambaqui gill powder as an iron source should be used with caution. On the other hand, iron in desiccated tambaqui liver powder is highly bioavailable, so other studies should assess the impact of adding this food to the diet of preschoolers and groups at risk of anemia, which would provide a new, alternative, and healthy source of dietary iron for Amazonians.

Conflict of Interests

The authors have not declared any conflict of interests.

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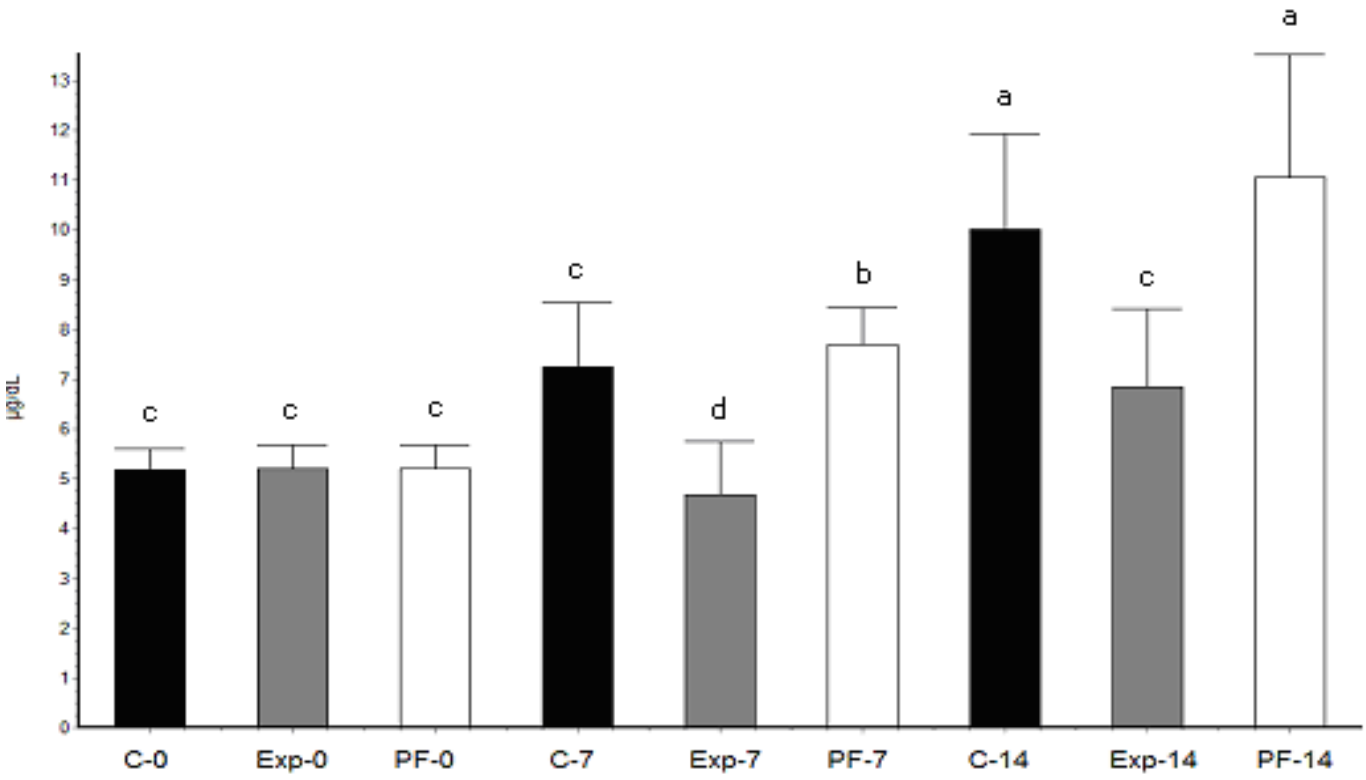

Figure 2. Hemoglobin levels (Hb) of animals in the control group (C), experimental group 2A (gills, Exp), and paired-feeding group (PF) at baseline (0), on day seven, and on day fourteen. Levels with the same letters are not significantly different according to the Tukey test ($p<0.05$).

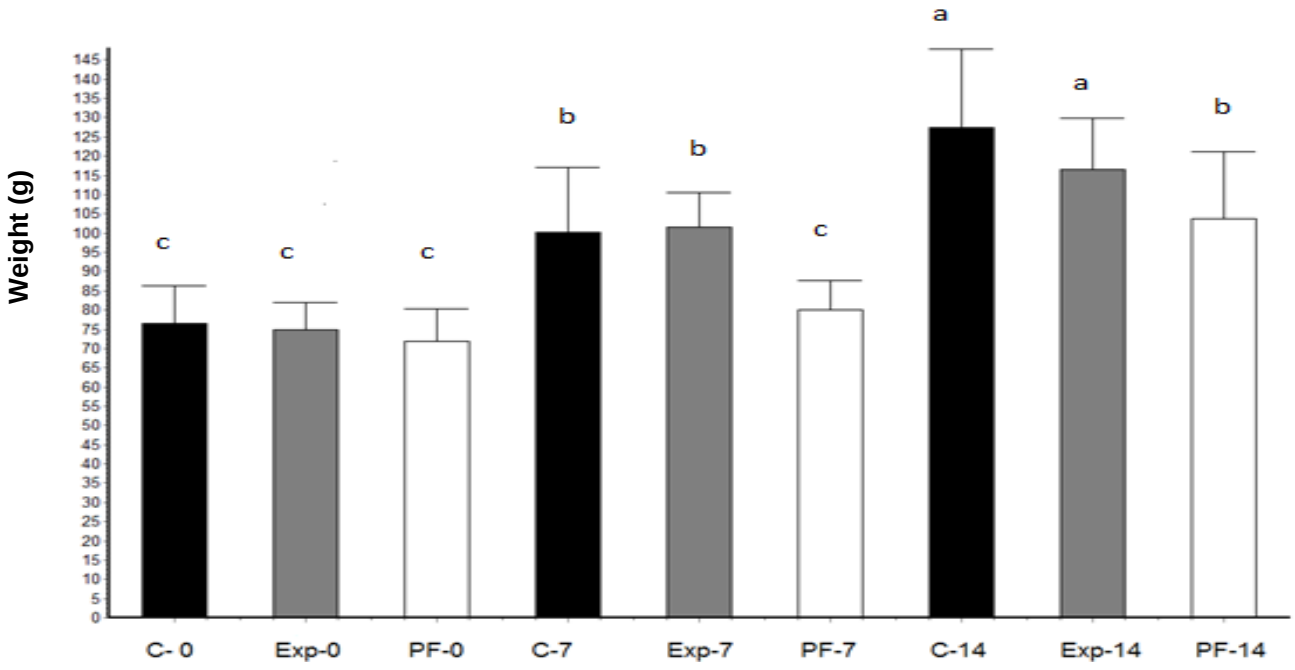

Figure 3. Weights of rats in the control group (C), experimental group 2 (liver, Exp), and paired-feeding group (PF) at baseline (0), on day seven, and on day fourteen. Weights with the same letters are not significantly different according to the Tukey test ($p<0.05$).

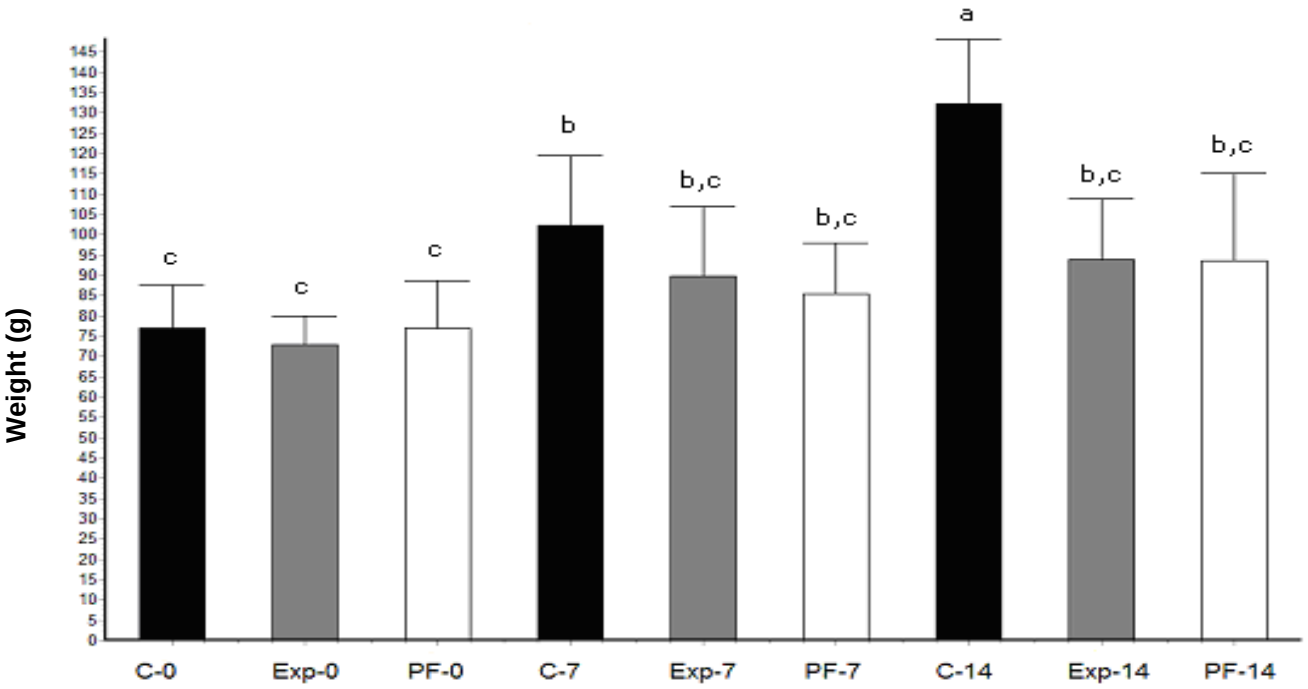

Figure 4. Weights of rats in the control group (C), experimental group 2A (gills, Exp), and paired-feeding group (PF) at baseline (0), on day seven (7), and on day fourteen (14). Weights with the same letters are not significantly different according to the Tukey test ($p<0.05$).

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