



## ANIMAL SCIENCE

# Effect of dietary caffeine supplementation on the carcass composition of pacu *Piaractus mesopotamicus*

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**Abstract:** Pacu (*Piaractus mesopotamicus*) is a fish with a high production potential in Brazil. However, one limitation is the excessive amount of ether extract in its carcass, an undesirable characteristic for the consumer. One approach to overcome this limitation is to improve carcass quality through zootechnical additives such as caffeine. The aim of this study was to evaluate the effect of supplementing the diet of pacu with caffeine on cut yield, biological indices, and carcass composition. Two hundred pacu with an initial weight of 1,687 g were used. The animals were allocated to 20 aquaculture cages of 1 m<sup>3</sup>, with 10 animals per cage. A completely randomized design with four treatments and five replicates was used. The treatments evaluated consisted of four inclusion levels of caffeine: T1 = 0.00 g; T2 = 0.16 g; T3 = 0.32 g, and T4 = 0.48 g caffeine.kg<sup>-1</sup> of feed. The findings show that caffeine can be recommended as a diet supplement for carcass improvement of pacu, reducing the fat content and increasing the protein content of the carcass. Caffeine up to 0.32 g.kg<sup>-1</sup> of feed can be added to the diet of pacu without affecting its performance or cut yield.

**Key words:** aquaculture, animal nutrition, body composition, fat content, trimethylxanthine, zootechnical additives.

## INTRODUCTION

Zootechnical additives have been used in animal feed (Lucio et al. 2021) as important tools to improve aquaculture. The goals are varied and include the stimulation of consumption, an increase in digestibility, and improvement of carcass composition (Ajiboye et al. 2012, Dias & Santigosa 2023). Within this context, researchers found that the inclusion of caffeine in the diet of rats (Franco et al. 2011) and sheep (Salinas-Rios et al. 2014) affects the carcass composition of these animals.

Caffeine (1,3,7-trimethylxanthine) is an alkaloid that belongs to the class of xanthines (Horrigan et al. 2006). The latter are derived from

purine nitrogenous bases. Caffeine is present in dietary supplements, nutraceuticals, medicines, and human foods. The biological effect of this compound is the result of its action on several molecular targets. Studies with different species have shown that caffeine has lipolytic effects, acting as a fatty acid mobilizer (Sinclair & Geiger 2000, Dangol et al. 2017); in addition, it may reduce intramuscular fat reserves and prevent hepatic lipid accumulation (Baldissera et al. 2019). In fact, studies have demonstrated a reduction in carcass fat content in finishing pigs (Parra et al. 2008) and flattening of the lobules of adipose tissue in male pigs (Chorilli et al. 2005).

Regarding species for aquaculture, the pacu (*Piaractus mesopotamicus*) is one of the most produced fish in Brazil (IBGE 2021). Its production has gained importance because of its large potential for intensive farming since the animals are easily adapted to captive breeding (Portella et al. 2014). However, its appreciation by consumers has declined because of the excessive presence of ether extract in its carcass, which ranges from 8 to 17% (Guinazi et al. 2006, Furuya et al. 2008). Moreover, excess lipid in its abdominal and visceral cavity leads to changes in body composition, nutritional value, final yield, and the storage stability of the produced fish (Bressan 2000).

Interest in the use of caffeine has increased because studies have shown that it improves the performance of juvenile Nile tilapia (Vieira et al. 2019). Therefore, this study assessed the effect of the use of different doses of caffeine as a zootechnical additive on the development and carcass composition of pacu.

## MATERIALS AND METHODS

### Location and animal ethics

The experiment was conducted at the Laboratory of Nutrition and Production of Ornamental Species (LNPEO), Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo (IFES), Campus Alegre. All procedures were previously approved by the Animal Ethics Committee of IFES, under protocol number 23149.00018411.794.

### Fish and experimental conditions

Two hundred apparently healthy pacu from the same batch, with an initial mean weight of  $1,687.1 \pm 359$  g, were used. The experiment lasted 35 days. A completely randomized design with four treatments and five replicates was used, totaling 20 experimental units. The experimental units consisted of  $1.0 \text{ m}^3$  aquaculture cages equipped

with feeders and lids. The cages were allocated to a fish hatchery measuring  $2,816.0 \text{ m}^2$ , with a mean depth of 3.0 meters. Apertures enabled the passage of water inside and outside. The animals were randomly assigned to the experimental units, with a density of 10 animals each.

### Preparation of experimental diet and feeding

Four experimental diets containing different doses of USP anhydrous caffeine ( $\text{C}_8\text{H}_{10}\text{N}_4\text{O}_2$ ) were prepared, with 99% purity. The diets consisted of commercial feed with 32% crude protein (Alinutre, Brazil) and the following caffeine doses: T1 = 0.00; T2 = 0.16; T3 = 0.32, and T4 =  $0.48 \text{ g.kg}^{-1}$  of feed. Caffeine was added to the feed using concrete mixers. For each 25 kg of feed, 1% fish oil and the amount of caffeine according to each treatment were added. The animals were fed ad libitum three times a day.

### Parameters of water quality

The physicochemical parameters of water were monitored throughout the experimental period. The pH, temperature, and dissolved oxygen were measured with a YSI 6920 V2<sup>®</sup> multiparameter probe (Yellow Springs Incorporated - YS, Yellow Springs, OH, USA) and were  $9.0 \pm 0.25$ ,  $22.31 \pm 1.97$  °C and  $6.8 \pm 0.85 \text{ mg.L}^{-1}$ , respectively. Total ammonia was determined based on descriptive methodology using standard methods for the examination of water and wastewater (Eaton & Franson et al. 2005) and was  $0.16 \text{ mg.L}^{-1} \pm 0.012$ .

### Animal performance

At the end of the experimental period, five animals per treatment were fasted for 24 hours and then euthanized with benzocaine hydrochloride diluted in aqueous solution. After euthanasia, the final biometrics of each individual were determined using a fish-measuring device. Height, total length, and

standard length were measured. The animals were weighed on a digital scale (UPX Acqua 15).

### Cutting yields and biological indices

The animals were decapitated and the weight of the head was obtained. The branchial arch, abdominal serrature, and viscera were removed. The viscera were weighed on a digital analytical scale (Ohaus Adventurer ARD110 4,100 g x 0.01 g Toledo) and the liver and coelomic fat were then separated and weighed on the same scale. The loin, rib (both sides), and tail were cut and weighed individually on a digital scale (UPX Acqua 15).

The following yield and biological indices were obtained: yield of gutted fish (YF): [fish weight without viscera (g)/final weight (g)] × 100; yield of fish loin (YL): [loin weight (g)/final weight (g)] × 100; yield of fish ribs (YR): [ribs weight (g)/final weight (g)] × 100; yield of fish tail (YT): [tail weight (g)/final weight (g)] × 100; viscerosomatic index (VSI): [viscera weight (g)/final weight (g)] × 100; hepatosomatic index (HSI): [liver weight (g)/final weight (g)] × 100; visceral fat index (VFI): [visceral fat weight (g)/final weight (g)] × 100.

### Bromatological composition

For the analysis of bromatological composition, the loin was collected from five animals per treatment and moisture, ash, lipids (Soxhlet method), and protein (Kjeldahl method) were determined. First, the loins were crushed in meat grinders and dried in an oven at 105°C for 24 hours to remove moisture from the samples. The samples were analyzed in triplicate using the standard methods of AOAC (2005).

### Statistics

The data were submitted to analysis of variance (ANOVA) and the Shapiro-Wilk normality test. Regression equations were estimated to describe the variables. The Tukey test was used for all variables under the study ( $p < 0.05$ ).

## RESULTS

### Animal performance and feed intake

Table I shows the mean values and models of animal performance. Animals that received higher levels of caffeine (0.32 and 0.48 g.kg<sup>-1</sup> of feed) exhibited a reduction in feed intake. Quadratic effects were observed for height,

**Table I. Zootechnical parameters (mean) of Pacu fed with different levels of caffeine. SD = standard deviation.**

Variables	Levels of caffeine (g.kg <sup>-1</sup> )				SD (g.kg <sup>-1</sup> )
	0	0.16	0.32	0.48	
Initial weight (g) <sup>ns</sup>	1,711.10	1,719.40	1,607.90	1,659.90	30.87
Final weight (g) <sup>A</sup>	1,697.60	1,502.80	1,571.20	2,028.4	19.80
Height (cm) <sup>B</sup>	16.36	16.32	16.52	17.46	4.86
Total length (cm) <sup>ns</sup>	43.10	41.44	42.08	44.77	6.92
Standard length (cm) <sup>C</sup>	37.54	35.48	37.68	39.08	5.55
Feed intake (g)*	2,644.21 <sup>a</sup>	2,602.61 <sup>a</sup>	2,330.41 <sup>b</sup>	2,307.04 <sup>b</sup>	56.21

\* Means with different letters on the same line differ according to ANOVA and Tukey's test ( $p < 0.05$ ); <sup>ns</sup> there were no differences between treatments according to ANOVA tests and regression; <sup>A</sup> Final weight:  $Y = 6,164x^2 - 2,309.5x + 1,707.2$  ( $R^2 = 0.3717$ ) ( $p$ -value = 0.0180); <sup>B</sup> Height:  $Y = 9.5703x^2 - 2.40625x + 16.385$  ( $R^2 = 0.341436$ ) ( $p$ -value = 0.0287); <sup>C</sup> Standard length:  $Y = 33.789x^2 - 11.95620x + 37.287$  ( $R^2 = 0.33$ ) ( $p$ -value = 0.0360).

standard length, and final weight, with a minimum point of 0.13 g.kg<sup>-1</sup>, 0.18 g.kg<sup>-1</sup> and 0.19 g.kg<sup>-1</sup> of levels of caffeine, respectively.

### Cutting yields, biological indices and bromatological composition

In general, caffeine did not change the carcass yield or biological indices of pacu (Table II). However, the addition of caffeine had a significant effect on tail yield.

The groups treated with caffeine had a higher concentration of mineral matter than the control group, with caffeine exerting a quadratic effect (Table III). Protein content was significantly higher in animals fed the diet with caffeine at the doses of 0.16, 0.32, and 0.48 g.kg<sup>-1</sup> compared to animals receiving feed without caffeine (Table III). The addition of caffeine to the diet reduced the ether extract content, which represents the amount of fat present in the animals' loin.

## DISCUSSION

Studies have shown that the addition of high doses of caffeine reduces animal performance (Ulloa & Verreth 2002, Vieira et al. 2019). In our study, when we evaluated the performance of

pacu, the addition of caffeine at the doses of 0.16 and 0.32 g.kg<sup>-1</sup> of feed caused weight loss. Moreover, lower feed intake was observed at higher inclusion levels of caffeine (0.32 and 0.48 g.kg<sup>-1</sup> of feed). A similar result was reported for fingerlings of tilapia (*Oreochromis niloticus*) fed diets containing caffeine doses higher than 0.33 g.kg<sup>-1</sup> of feed (Vieira et al. 2019).

Caffeine has a bitter taste (Chatzifotis et al. 2008) that can reduce feed palatability. Therefore, considering the findings of this study and the literature data, we believe that the weight loss was caused by the reduction in feed intake observed in the groups fed high doses of caffeine due to the bitter taste (Chatzifotis et al. 2008). Caffeine exerted quadratic effects on final weight, standard length, and height, with minimum points of about 0.13 g.kg<sup>-1</sup>. The use of caffeine as a feed additive in pacu can be recommended in the final stages of production and is feasible as long as it respects the limit of addition.

The effect of caffeine and of repartitioning agents has been studied in different species. Hwang et al. (2012) showed that the use of green tea extract, a caffeine-rich substance, produces

**Table II. Carcass composition and biological indices (mean) of Pacu fed diets with different levels of caffeine. SD = standard deviation.**

Variables (%)	Levels of caffeine (g.kg <sup>-1</sup> )				SD (g.kg <sup>-1</sup> )
	0	0.16	0.32	0.48	
Hepatosomatic index <sup>ns</sup>	1.65	1.26	1.25	1.12	0.27
Visceral fat index <sup>ns</sup>	2.23	2.39	2.70	3.42	0.57
Eviscerated fish yield <sup>ns</sup>	90.72	92.56	93.45	92.18	2.37
Loin yield <sup>ns</sup>	22.87	23.76	21.09	20.64	15.19
Rib yield <sup>ns</sup>	33.71	32.21	29.89	27.95	15.25
Tail yield <sup>A*</sup>	17.87 <sup>a</sup>	18.72 <sup>a</sup>	15.52 <sup>b</sup>	14.74 <sup>b</sup>	0.14
Viscerosomatic index <sup>ns</sup>	9.28	7.44	6.55	7.81	0.28

\* Means with different letters on the same line differ according to ANOVA and Tukey's test ( $p < 0.05$ ); <sup>ns</sup> there were no differences between treatments according to ANOVA tests and regression; <sup>A</sup>Tail yield:  $Y = -15.776x^2 - 0.2781x + 18.196$  ( $R^2 = 0.2943$ ) ( $p$ -value = 0.0516).

**Table III. Bromatological composition (mean) of Pacu loin fed diets with different levels of caffeine. SD = standard deviation.**

Variables (%)	Levels of caffeine (g.kg <sup>-1</sup> )				SD (g.kg <sup>-1</sup> )
	0	0.16	0.32	0.48	
Moisture <sup>ns</sup>	75.63	75.66	76.24	76.11	0.94
Dry matter <sup>ns</sup>	24.37	24.35	23.35	23.89	2.97
Ash <sup>A*</sup>	1.40 <sup>a</sup>	1.42 <sup>b</sup>	1.45 <sup>b</sup>	1.46 <sup>b</sup>	0.13
Crude protein <sup>B*</sup>	63.22 <sup>a</sup>	69.35 <sup>b</sup>	69.88 <sup>b</sup>	73.28 <sup>b</sup>	3.62
Ether extract <sup>C*</sup>	22.63 <sup>a</sup>	20.25 <sup>a</sup>	16.39 <sup>b</sup>	11.80 <sup>b</sup>	1.92

\* Means with different letters on the same line differ according to ANOVA and Tukey's test ( $p < 0.05$ ); <sup>ns</sup> there were no differences between treatments according to ANOVA tests and regression. <sup>A</sup> Ash:  $Y = -31.92x^2 + 26.87x + 13.94$  ( $R^2 = 0.59904$ ) ( $p$ -value = 0.01637); <sup>B</sup> Crude protein:  $Y = -26.56x^2 + 31.963x + 63.649$  ( $R^2 = 0.7081$ ) ( $p$ -value = 0.0039); <sup>C</sup> Ether extract:  $Y = -21.4777x^2 - 12.398x + 22.669$  ( $R^2 = 0.995$ ) ( $p$ -value = 0.0001).

a significant difference in the biological indices. The authors explain the results found by metabolic changes caused by the addition of this substance. In the present study, we identified no significant differences in the biological indices evaluated or in cut yield when purified caffeine was added to the diet. Ferreira et al. (2011) and Marinho et al. (2007) also found no change in cut yield (e.g., loin) when purified repartitioning agents such as caffeine and ractopamine were used. Taken together, the findings suggest that caffeine at doses of 0 to 48 g.kg<sup>-1</sup> of feed does not influence biological indices or carcass yield.

Few studies have investigated the effect of caffeine and its derivatives on fish body composition. In the present study, caffeine intake changed the composition of the loin of pacu. The addition of increasing doses of caffeine to the feed increased the concentrations of mineral matter and protein and reduced ether extract, in agreement with the results obtained for pigs (Chorilli et al. 2005), Sprague-Dawley rats (Kobayashi-Hattori et al. 2005), and juvenile pacu (*Piaractus mesopotamicus*) (Bicudo et al. 2012).

Caffeine can induce lipolysis of fatty acids in adipose tissue by activating  $\beta$ -adrenergic receptors, thus promoting the breakdown and

release of free fatty acids into the plasma (Kim et al. 2010). Based on the results found in this study and on the already known lipolytic action of caffeine in other species (Chorilli et al. 2005, Kobayashi-Hattori et al. 2005), we believe that caffeine may activate lipase in tissues of pacu, reducing the concentration of ether extract in their loins. Furthermore, caffeine favors the deposition of protein tissue over adipose tissue. Therefore, the present results show that the use of caffeine as an additive is effective in reducing ether extract in pacu.

In conclusion, the use of caffeine as an additive may be recommended for pacu in order to reduce fat content and to increase protein content of the carcass in the final production stages. Caffeine up to 0.32 g.kg<sup>-1</sup> of feed can be added to the pacu diet without affecting the performance or cut yield of these animals.

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