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Effect of feeding restrictions on development of juvenile cobias, *Rachycentron canadum*

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Abstract

Aim of study: A 45-day trial was conducted to evaluate fasting as a possible way of food strategy during production of juvenile cobias (*Rachycentron canadum*).

Area of study: The study was conducted in the State of Espírito Santo, Brazil.

Material and methods: The following different protocols were used to organize their feeding: C, fish fed to satiety twice a day for 45 days; U1, fish fed to satiety twice a day on alternate days; U2, fish fed to satiety twice daily for five consecutive days, followed by two days of food deprivation, cyclically; U5, fish subjected to food deprivation for five days and then fed to satiety twice a day for 40 days; U10, fish subjected to food deprivation for ten days and then fed to satiety twice a day for 35 days; and U15, fish subjected to food deprivation for fifteen days and then fed to satiety twice a day for 30 days.

Main results: No mortality was observed during the trial period. The different feeding protocols significantly affected juvenile cobias development and wellbeing. The final weight (g) of cobia juveniles was C – 91.9 ± 9.1; U1 – 75.0 ± 11.2; U2 – 72.2 ± 6.0; U5 – 70.3 ± 6.1; U10 – 63.4 ± 4.6; U15 – 54.4 ± 4.7. No compensatory growth was observed during the entire experimental period.

Research highlights: Continuous fasting had a more severe effect than intermittent fasting. Significantly greater hepatocyte counts were correlated with longer fasting periods. Fasting protocols should not be recommended for juvenile cobias.

Additional key words: fasting; beijupirá; marine farming; condition factor; fish wellbeing; feeding protocols.

Abbreviations used: CF (condition fator); FCE (feed conversion efficiency); FI (dry feed intake); FID (daily feed consumption); HSI (hepatosomatic index); L0 (initial length); Lt (final length); LWR (liver-weight ratio); SGRL (length-specific growth rate); SGRW (weight-specific growth rate); VSI (viscerosomatic index); W0 (initial body weight); WG (weight gain); Wt (final body weight).

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Introduction

Due to the large variation in food availability in the natural environment, fish can suffer food deprivation, which causes variation in growth rates (Chappaz et al., 1996; Cavalli et al., 1997; Motta et al., 2021). In aquaculture, food deprivation can occur as a consequence of attempts to save on labor costs (Oh et al., 2013), environmental concerns (Cho et al., 2006) or unpredictable events, e.g. storms; wind (Dempster et al., 2002; Morro et al., 2021). Therefore, understanding the effect of fasting on fish would be of great interest to producers (Tunçelli & Pirhonen, 2021; Le François et al., 2023).

Ending food deprivation (resumption of feeding) can lead animals to present a phenomenon called compensatory growth. According to Ali et al. (2003), this consists of a phase of accelerated growth, until normal conditions are restored. This characteristic has already been observed in several animals, including fish.

The characteristics of compensatory growth are species-dependent, i.e. each species of fish exhibits behavior differing from the others (Tian & Qin, 2003). According to Ali et al. (2003), several studies have shown that compensatory growth can occur consequent to total or partial deprivation of food. This growth can occur in different ways: a) full compensation – the animals deprived of feeding manage to reach the same size as the control group after resumption of feeding; b) partial compensation – the animals are unable to reach the same size, but show accelerated growth rates or better feed conversion rates; or c) overcompensation – the animals reach a larger size than those fed normally.

The cobia (*Rachycentron canadum*) was first described in 1766 by Linnaeus as *Gasterosteus canadus* and gained its current name after several taxonomic revisions. It is a pelagic coastal fish species that can be found in tropical and subtropical waters around the world, except in the eastern Pacific Ocean (Shaffer & Nakamura, 1989). It is a fast-moving carnivorous fish (Ditty & Shaw, 1992; Fraser & Davies, 2009) that feeds on crustaceans, fish and squid (Franks et al., 1996).

It presents rapid growth of 4 to 6 kg per year, with excellent feed conversion rates, and larviculture is highly successful (Chou et al., 2001). Moreover, it has good acceptance in the market. These features make this fish an excellent species for cultivation. However, despite the enormous potential of this fish, many studies still need to be carried out. One of the issues still to be resolved formed the objective of this study, which was to evaluate fasting as a possible way to obtain accelerated growth during cultivation and, to analyze the effect of fasting on the development of juveniles regarding periods of food deprivation resulting from unpredictable events.

Material and methods

Fish specimens (both males and females) were obtained from a specialized laboratory and had an average weight of approximately 15 g (Table 1). After undergoing a one-day acclimation process, the fish were homogenously distributed into the experimental units. In total, six treatments were performed, each with three replications, thus totaling eighteen experimental units. Each experimental unit received six fish and therefore a total of 108 fish were used. The experimental units were distributed in an entirely randomized design.

The experimental system involved closed continuous recirculation with mechanical and biological filtration. A centrifugal pump (BOYU spf-18000; 450 W) ensured a water flow of 110 L h^{-1} in each experimental unit. The 18 experimental units (glass aquariums of dimensions 50 × 50 × 50 cm and capacity 125 liters) were siphoned daily and the water parameters of dissolved oxygen (mg L^{-1}), temperature (ºC), ammonia (mg L^{-1}) and pH were monitored twice a day throughout the experimental period. These water quality parameters remained unchanged throughout the experimental period, with the following values: dissolved oxygen (5.61 ± 0.38 mg L^{-1}), temperature (26.7 ± 1.4 ºC), ammonia (0.12 ± 0.09 mg L^{-1}) and pH (7.83 ± 0.22).

The trial period was 45 days. The different protocols used for organizing the feeding were as follows: C, fish fed to satiety twice a day (09:00 and 16:00) throughout the experimental period; U1, fish fed to satiety twice a day (09:00 and 16:00) on alternate days during the 45 days of the experiment (total of 23 days of feeding); U2, fish fed to satiety twice daily (09: 00 and 16:00) for five consecutive days, followed by two days of food deprivation, cyclically during the 45 days of the experiment; U5, fish subjected to food deprivation for five days and then fed to satiety twice a day (09:00 and 16:00) for 40 days; (U10) fish subjected to food deprivation for ten days and then fed to satiety twice a day (09:00 and 16:00) for 35 days; and U15, fish subjected to food deprivation for fifteen days and then fed to satiety twice a day (09:00 and 16:00) for 30 days.

During the experimental period, the fish were fed with extruded feed (48.3 g/100g of crude protein; 13.33 g/100g of ether extract; and 11.8 g/100g of moisture). The diet was formulated with the following ingredients: flattened rice (20.0 g/100g), poultry byproducts (20.0 g/100g), soy protein concentrate (16.94 g/100g), corn (15.5 g/100g), meat meal (10.0 g/100g), soy protein (6.1 g/100g), fish meal (5.0 g/100g), marine fish oil (4.46 g/100g), vitamin premix (1.0 g/100g) and mineral premix (1.0 g/100g).

To control feed consumption, for each repetition a vessel was used that was initially weighed with its volume full of extruded feed. Daily (after the second feeding of the day), each vessel was weighed to control feed consumption. The vessels were stored in a refrigerator to maintain the quality of the extruded feed.

At the beginning of the experiment, all the fish were anesthetized with eugenol and then weighed on an analytical balance with an accuracy of 0.001 g and measured with the aid of a caliper. At that time, the livers and intestines of ten juveniles were collected for initial analyses of these organs.

Table 1. Zootechnical performance indices of cobia juveniles submitted to different feeding treatments (see text) and observed at different time points during the experimental period. Different letters in the same line represent significant differences between treatments.

Index	Day	Treatment					
		C	U1	U2	U5	U10	U15
Weight (g)	0	15.7 ± 1.4	15.5 ± 1.3	15.7 ± 1.4	15.7 ± 1.3	15.5 ± 1.5	15.6 ± 1.5
	15	37.3 ± 4.1^{a}	31.6 ± 2.5^{ab}	29.6 ± 3.7^{b}	27.7 ± 3.3^{bc}	20.7 ± 2.7^{cd}	13.5 ± 1.5^{d}
	30	63.0 ± 6.1^{a}	58.1 ± 8.6^{ab}	53.3 ± 3.8^{b}	51.0 ± 7.6^{b}	42.0 ± 6.7^{c}	32.8 ± 2.7^{d}
	45	91.9 ± 9.1^{a}	75.0 ± 11.2^{b}	72.2 ± 6.0^{b}	70.3 ± 6.1^{bc}	63.4 ± 4.6^{c}	54.4 ± 4.7^{d}
Weight gain (g)	15	21.5 ± 1.5^{a}	16.1 ± 0.5^{b}	14.0 ± 1.3^{c}	12.2 ± 1.2^{c}	5.0 ± 0.6^{d}	-2.3 ± 0.3^{e}
	30	25.7 ± 0.7	26.4 ± 3.3	23.5 ± 3.5	23.1 ± 3.3	21.2 ± 1.8	19.2 ± 1.7
	45	28.4 ± 7.4	16.8 ± 2.8	18.5 ± 4.9	19.0 ± 3.3	21.3 ± 1.7	21.3 ± 4.5
SGR_W (% day^{-1})	15	5.7 ± 0.3^{a}	4.7 ± 0.1^{b}	4.3 ± 0.3^{bc}	3.9 ± 0.3^{c}	1.8 ± 0.2^{d}	-1.1 ± 0.2^{e}
	30	3.5 ± 0.1^{b}	4.0 ± 0.3^{b}	3.9 ± 0.6^{b}	4.0 ± 0.5^{b}	4.7 ± 0.2^{b}	5.9 ± 0.4^{a}
	45	2.5 ± 0.5^{a}	1.7 ± 0.2^{b}	2.0 ± 0.5^{ab}	2.1 ± 0.4^{ab}	2.7 ± 0.1^{a}	3.3 ± 0.6^{a}
Length (cm)	0	14.7 ± 0.4	14.7 ± 0.3	14.7 ± 0.5	14.7 ± 0.5	14.6 ± 0.6	14.7 ± 0.6
	15	17.9 ± 0.4^{a}	17.5 ± 0.3^{a}	17.5 ± 0.5^{a}	16.5 ± 0.5^{b}	15.4 ± 0.6^{c}	15.0 ± 0.6^{c}
	30	21.4 ± 1.4^{a}	20.4 ± 0.9^{ab}	20.0 ± 0.8^{b}	19.7 ± 1.0^{b}	18.5 ± 0.9^{c}	17.1 ± 0.6^{d}
	45	23.3 ± 0.8^{a}	22.6 ± 0.5^{a}	22.3 ± 0.7^{ab}	21.4 ± 1.0^{bc}	20.9 ± 0.6^{cd}	20.0 ± 0.6^{d}
SGR_L (% day^{-1})	15	1.3 ± 0.1^{a}	1.2 ± 0.1^{a}	1.2 ± 0.1^{a}	0.8 ± 0.1^{b}	0.4 ± 0.1^{c}	0.2 ± 0.1^{c}
	30	1.2 ± 0.2^{ab}	1.0 ± 0.1^{ab}	0.9 ± 0.1^{ab}	1.2 ± 0.2^{ab}	1.2 ± 0.2^{a}	0.9 ± 0.1^{b}
	45	0.6 ± 0.2^{b}	0.7 ± 0.1^{b}	0.7 ± 0.3^{ab}	0.6 ± 0.2^{b}	0.8 ± 0.1^{ab}	1.0 ± 0.2^{a}
FI (g)	15	95.7 ± 1.7^{a}	75.6 ± 3.2^{b}	67.7 ± 5.1^{b}	55.9 ± 3.8^{c}	23.6 ± 1.7^{d}	0
	30	89.8 ± 7.0^{a}	72.9 ± 7.8^{b}	61.5 ± 7.2^{bc}	63.8 ± 2.5^{bc}	67.6 ± 7.8^{bc}	60.6 ± 4.9^{c}
	45	52.8 ± 5.2	44.1 ± 2.5	40.9 ± 4.9	45.4 ± 2.5	44.2 ± 1.8	45.4 ± 4.4
FID (g day^{-1})	15	6.4 ± 0.1^{b}	9.5 ± 0.4^{a}	6.2 ± 1.0^{b}	5.6 ± 0.4^{bc}	4.7 ± 0.3^{c}	0
	30	6.0 ± 0.5^{b}	10.4± 1.1^{a}	5.6 ± 1.4^{bc}	4.3 ± 0.2^{cd}	4.5 ± 0.5^{cd}	4.0 ± 0.3^{d}
	45	3.5 ± 0.3^{b}	5.5 ± 0.3^{a}	3.7 ± 1.0^{b}	3.0 ± 0.2^{b}	3.0 ± 0.1^{b}	3.0 ± 0.3^{b}
FCE (%)	15	22.5 ± 1.8	21.3 ± 1.0	20.7 ± 0.4	21.8 ± 0.6	21.3 ± 1.7	0
	30	28.7 ± 1.6	36.4 ± 1.2	39.3 ± 10.8	36.7 ± 6.5	32.0 ± 6.5	31.8 ± 0.6
	45	54.5 ± 9.4	38.6 ± 8.4	45.7 ± 6.8	42.6 ± 8.7	48.3 ± 3.7	47.5 ± 7.3
VSI (%)	15	20.1 ± 0.9^{b}	18.9 ± 1.6^{bc}	16.0 ± 1.7^{c}	20.3 ± 1.3^{b}	25.8 ± 0.4^{a}	10.8 ± 1.0^{d}
	30	20.0 ± 1.7^{b}	22.7 ± 0.5^{ab}	23.6 ± 0.7^{ab}	16.1 ± 0.3^{c}	21.1 ± 0.2^{ab}	23.7 ± 2.8^{a}
	45	18.9 ± 1.4	18.2 ± 0.6	20.1 ± 2.4	18.4 ± 0.8	19.2 ± 0.6	19.9 ± 0.8
HSI (%)	15	7.2 ± 0.2^{a}	6.4 ± 0.5^{ab}	5.6 ± 0.8^{b}	6.9 ± 0.2^{ab}	6.2 ± 0.1^{ab}	2.6 ± 0.3^{c}
	30	6.4 ± 0.1^{ab}	6.0 ± 0.4^{ab}	5.8 ± 0.4^{b}	5.7 ± 0.4^{b}	7.3 ± 0.1^{ab}	7.6 ± 0.2^{a}
	45	7.1 ± 0.2	6.3 ± 0.4	7.1 ± 0.5	6.7 ± 0.7	7.8 ± 0.6	7.6 ± 0.5
CF	0	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.22 ± 0.01
	15	0.26 ± 0.01^{a}	0.24 ± 0.01^{bc}	0.23 ± 0.01^{c}	0.25 ± 0.01^{ab}	0.24 ± 0.01^{b}	0.17 ± 0.01^{d}
	30	0.26 ± 0.01^{a}	0.24 ± 0.01^{b}	0.23 ± 0.01^{b}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}
	45	0.28 ± 0.01^{a}	0.25 ± 0.01^{b}	0.25 ± 0.01^{b}	0.28 ± 0.01^{a}	0.27 ± 0.01^{a}	0.27 ± 0.01^{a}
LWR	15	1.17 ± 0.04^{a}	1.61 ± 0.04^{a}	1.89 ± 0.04^{a}	1.39 ± 0.04^{a}	1.65 ± 0.04^{a}	2.68 ± 0.04^{b}
	30	1.95 ± 0.04^{a}	2.53 ± 0.04^{a}	2.53 ± 0.04^{b}	2.23 ± 0.04^{a}	1.46 ± 0.04^{a}	1.35 ± 0.04^{a}
	45	2.04 ± 0.04^{a}	1.88 ± 0.04^{a}	3.09 ± 0.04^{b}	2.03 ± 0.04^{a}	1.72 ± 0.04^{a}	1.63 ± 0.04^{a}

After 15, 30 and 45 days of the experiment, all the fish were weighed and measured. At each weighing, two fish from each experimental unit were necropsied to obtain the weight of the viscera and liver. These were firstly anesthetized and were then sacrificed by means of sectioning of the spinal cord immediately posterior to the occipital region.

The growth and feed utilization parameters were calculated as follows: weight gain WG (g) = Wt – W0;

weight-specific growth rate SGR_W (% day^{-1}) = (lnWt − lnW0) × 100/t; length-specific growth rate SGR_L (% day^{-1}) = (lnLt − lnL0) × 100/t; feed conversion efficiency FCE (%) = 100*(wet weight gain (g)/FI (g)); daily feed consumption FID (FI/days of feeding); viscerosomatic index VSI (%) = (visceral weight (g)/body weight (g)) × 100; and hepatosomatic index HSI (%) = (liver weight (g)/body weight (g)) × 100; where W0 and Wt are the initial and final body weights of the fish, t (days) is the duration of the experiment; L0 and Lt (cm) are the initial and final lengths of the fish; FI is the dry feed intake (g).

The weight-length ratio of the fish was expressed in terms of the equation proposed by Pauly (1984): $W = aL^b$. The linear transformation proposed by Zar (1984) was then used, following the equation: Log W = b log L + log a; where "W" is the weight (g) of the fish, "L" is the total length (cm) of the fish, "a" is the exponent describing the rate of change of weight with length (intercept) and "b" is the weight per unit length (slope).

To obtain data on fish wellbeing, the condition factor (CF) and the liver-weight ratio (LWR) were calculated (Gomiero & Braga, 2005; Omogoriola et al., 2011). The condition factor is represented by the letter "K" when fish are measured and weighed, following the equation proposed by Pauly (1984): $K = 100W/L^b$; where K is the condition factor, W is the weight (g) of the fish, L is the total length (cm) of the fish and b is the value obtained from the length-weight equation. For the CF, this value "K" can basically be interpreted as "higher values represent better welfare condition"; and for LWR, "higher values show that the weight of the liver is decreasing at a higher rate than the body of the animal".

The analysis was performed using the theory of generalized linear models for the variables SGR_W, SGR_L, FCE, VSI, and HSI. The normal, lognormal, exponential and gamma distributions were evaluated using the GLIMMIX procedure of the Statistical Analysis System software (SAS System, Inc., Cary, NC, USA) (Stokes et al., 1995). The distribution was chosen using the Akaike criterion (Sugiura, 1978). After choosing the most plausible distribution, the Tukey test was applied when significance was observed (5%).

For the characteristics of weight, weight gain, length, FI, and FID, the analysis was performed using the theory of mixed linear models, using the PROC MIXED procedure of the SAS software (SAS System, Inc., Cary, NC, USA) (Stokes et al., 1995), and the Tukey test was applied when significance was observed (5%).

The livers were fixed in 10% buffered formalin for one week. They were then dehydrated in an increasing series of ethyl alcohol, clarified with xylol and, lastly, embedded in paraffin, following the routine histopathological techniques (Motta et al., 2021). Serial sections of 5 µm in thickness were obtained with the aid of a microtome and these were then subjected to routine dewaxing, hydration and staining techniques (Humason, 1972). Subsequently, the histological slides were analyzed under an optical microscope (Leica DM500) and were photographed using a camera coupled to this microscope. The degree of vacuolization was quantified by counting hepatocytes, per maximum area visible when looking through the microscope eyepiece at each microscope field of view (100X), per histological slide, using the Image J software (Schneider et al., 2012). The relationship observed related to the increase in the volume of hepatocytes when accumulation of energy reserves occurred, and the consequent decrease in the number of hepatocytes possibly observed in one field of view of the microscope, as adapted from Motta et al. (2021).

Results

No mortality was observed during the trial period. The different feeding protocols significantly affected ($p < 0.05$) the development of juvenile cobias. The zootechnical performance data can be seen in Table 1. The data obtained in the present experiment on feed intake did not allow us to conclude that there was hyperphagia in any of the treatments.

By the end of the 45-day experiment, the animals that had been subjected to the U2 feeding protocol had been fed on 33 days, while those that had been subjected to the U10 feeding protocol had been fed on 35 days. However, it was observed that the final weight of the U2 fish was greater than that of the U10 fish. The 15-day uninterrupted fast caused loss of mass for the juvenile cobias.

In the histological analysis, it could be seen that the nucleus of the hepatocytes was either imperceptible or displaced to the extremities. Intense vacuolization of hepatocytes was also observed (Fig. 1).

Significantly increased ($p < 0.05$) hepatocyte counts were correlated with longer fasting periods (Fig. 2). In the first 15 days of the experiment, the fish that were subjected to treatments involving uninterrupted fasting (U5, U10 and U15) presented higher hepatocyte counts ($p < 0.05$) than the fish subjected to the control (C) and intermittent fasting (U1) treatments (Fig. 2). In the continuous fasting treatments, after periods of 30 and 45 days of refeeding, the fish presented hepatocytes with greater vacuolization, but no normalization was observed in comparison with what was observed from the control treatment ($p < 0.05$).

Discussion

Cobia farming is carried out in open-ocean submerged cages (Benetti et al., 2010). In these aquaculture systems, abiotic factors (e.g. storms, temperature variations or wind) may result in fasting or low efficiency of food use (Morro et al., 2021). Nonetheless, in the present experiment conducted in aquariums, the survival data, recovery of energy reserves and condition factor were indicative of the resilience and phenotypic plasticity of this species.

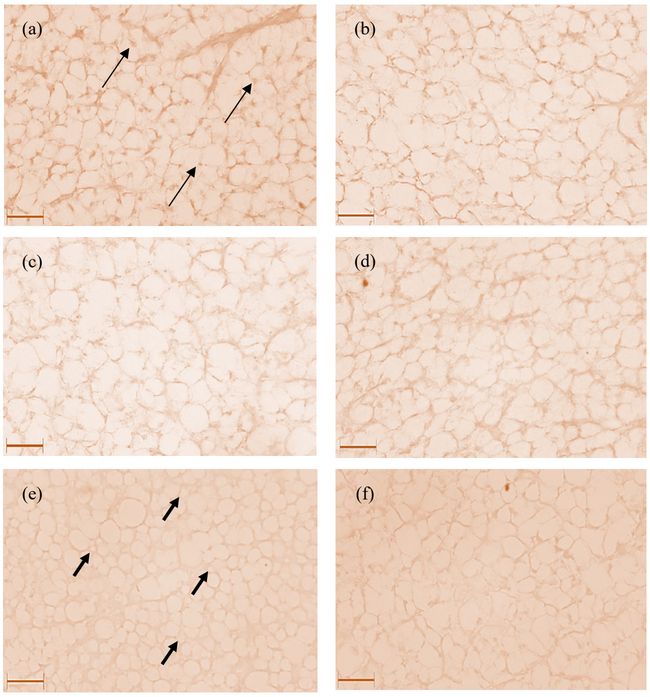

Figure 1. Liver images of cobia (*Rachycentron canadum*) subjected to different feeding treatments: (a) control, 0 days; (b) control, 15 days; (c) control, 30 days; (d) U5, 15 days; (e) U15, 15 days; (f) U15, 30 days. Thin arrow: hepatocytes with nucleus displaced to the periphery, indicating hepatic steatosis; thick arrow: low-volume hepatocytes - hepatocytes with reduced volume - it is possible to observe a greater number of hepatocytes in the microscope field of view. Scale bar: 200 µm

However, no compensatory growth was observed at any stage of the experiment. Similar results were observed by Tunçelli & Pirhonen (2021) for rainbow trout (*Oncorhynchus mykiss*), Motta et al. (2021) for the freshwater angelfish (*Pterophyllum scalare*) and Peres et al. (2011) for *Sparus aurata*. In all these cases, fasting did not give rise to an interesting alternative feeding protocol, and there was even the possibility of occurrence of weight loss.

Hyperphagia is one of the events that, after feedback, has been indicated as responsible for compensatory growth (Ali et al., 2003). In the present experiment, the intermittent fasting of one day of feeding followed by one day of fasting (U1) increased the daily feed consumption, but in the other treatments with fasting, no significant increase in daily feed consumption was observed. Thus, one of the factors that may have affected the non-observation of compensatory growth in the present experiment was the non-observation of hyperphagia.

On the other hand, Hvas et al. (2022) worked with Atlantic salmon (*Salmo salar*) and observed that, despite the loss of mass among these fish during the eight-week fasting period, five weeks of refeeding was sufficient for them to regain their expected weight. This ability to recover from a period of fasting is directly related to the phenotypic plasticity of the species.

Regarding phenotypic plasticity, in the present experiment, juvenile cobias that underwent 15 consecutive days of fasting presented a higher number of hepatocytes per microscope field of view. This result is a consequence of a lower degree of vacuolization of hepatocytes, which causes each liver cell to occupy less space (smaller volume), thus increasing the total number of cells per field of view in the microscope. A lower degree of vacuolization of hepatocytes is an indication of mobilization of energy reserves of the liver. According to Qian et al. (2016), accessing energy reserves by using metabolic pathways such as lipolysis and glycolysis/gluconeogenesis is one of the responses of the fish's metabolism to a period of food deprivation.

Furthermore, the values observed for the liver-weight relationship in the present experiment indicate that the weight of the liver decreases more rapidly than the weight of the animal. These data corroborate the histology data observed, as mentioned above, which is another indicator that for juvenile cobias, the liver provides an important energy reserve for use during fasting periods. The changes experienced by fish when fasting can also be observed through the VSI and HSI variables, given that these can undergo significant decreases after certain fasting periods, as observed by Turano et al. (2007) and Tunçelli & Pirhonen (2021). The results obtained by Tunçelli & Pirhonen (2021) in their study on rainbow trout (*O. mykiss*) and by Känkänen & Pirhonen (2009) in their study on European whitefish (*Coregonus lavaretus*) corroborate the hypothesis that fish adapt when subjected to long-term fasting protocols.

The degree of depletion of the energy reserves of the liver seems to be linked to the duration of food deprivation. There is a correlation between increases in the fasting period and decreased hepatocyte vacuolization. This situation, observed in the present experiment, had already been reported by Gaucher et al. (2012) in relation to *Hyphessobrycon luetkenii* and by Motta et al. (2021) in relation to juvenile freshwater angelfish (*P. scalare*).

In contrast, Macêdo et al. (2020), who worked with Nile tilapia (*Oreochromis niloticus*), did not observe any influence from one and two-day intermittent fasting on hepatocyte vacuolization. It is possible that these authors did not observe any significant difference in their histological analysis on the livers of these fish because of the short fasting time to which they had been subjected. Information regarding the severity of the depletion of energy reserves is essential for understanding the extent of the damage caused by food deprivation, and is also important with regard to animal recovery surveys.

Along this line of reasoning, data on the condition factor are relevant because they are indicative of the well-being of the fish. In the present experiment, data on this variable showed that fish that underwent periods of fasting

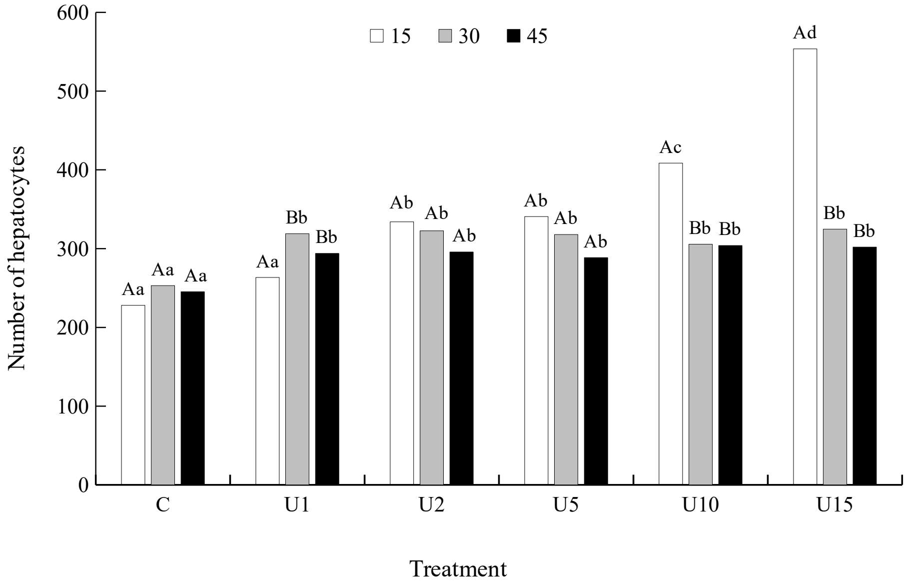

Figure 2. Number of hepatocytes observed in the histological slides of livers of juvenile cobias submitted to different feeding protocols. For all treatments, samples from three different experimental periods (15, 30, and 45 days) were analyzed. Correlations were made between the treatments at the same time points and within treatment at different time points. Lowercase letters refer to the comparison between treatments within the same period (15, 30, or 45 days); uppercase letters refer to the comparison of different periods within the same treatment. Different letters represent significant differences ($p < 0.05$).

suffered but presented recovery after feeding was resumed. Regarding recovery, fish that were subjected to continuous fasting, especially 10 and 15 days of uninterrupted fasting, seemed to suffer more than those that underwent intermittent fasting. It is likely that after reaching a certain duration of fasting, fish end up suffering anatomical and physiological changes of greater severity. Longer times would be required for recovery from such situations. Working with the freshwater angelfish (*P. scalare*), Motta et al. (2021) observed that juveniles of this species that were subjected to 15 days of fasting needed more than 30 days of refeeding to recover, while those that underwent 12 days of fasting completely recovered their liver reserves with 30 days of refeeding.

Although continuous prolonged fasting was found in the present study to affect juvenile cobias more severely, it is important to mention that fish that underwent intermittent fasting treatments presented stress throughout the experimental period. This was seen from the values of the condition factor and liver-weight ratio. Therefore, considering that fasting causes loss of performance and does not lead to compensatory gain, fasting should not be recommended for juvenile cobias as a feeding protocol. Nonetheless, the data indicate that fish welfare became restored after fasting, and there was no mortality. This allows us to conclude that in fasting situations caused by unpredictable events (e.g. storms or wind), these fish can resist until refeeding becomes possible.

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