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Communication

[*Comunicação*]

The effect of feeding frequency on the performance of juvenile cobia (*Rachycentron canadum*)

[*Efeito da frequência alimentar no desempenho de juvenis do cobia* (Rachycentron canadum)]

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Mariculture is an expanding activity that has advanced from a total production of approximately 9.2 million tons in the 1990s to 33.1 million tons in 2020 (FAO, 2022). Despite this growth, marine species (37.83%) account for a smaller share of this production than freshwater species (62.17%) (FAO, 2022). Among the marine species with potential for aquaculture, the cobia (*Rachycentron canadum*) is a species that has excellent performance indices, such as rapid growth (4.0 to 6.0kg per year) and feed conversion (1.0 to 1.8), and larviculture is successfully performed (Zhang *et al*., 2021).
Several factors, *e.g.*, production systems (Bergamo *et al*., 2021), nutrition and feeding management (Gilannejad *et al*., 2019; Zhang *et al*., 2021), and fish heath (Chen *et al*., 2001) affect mariculture and therefore this sector does not present results as expressive as in other aquaculture sectors. Among such factors, lack of knowledge about feeding management among marine species is a serious problem since food is currently the main cost in animal production (Baki and Yücel, 2017; Ekmekçi and Gül, 2017).

If the feeding frequency, which can be defined as the number of times animals receive food in a day, is properly established, a state of increased efficiency of use of food by animals can be reached (Gilannejad *et al*., 2019). Furthermore, the feeding frequency is directly related to other variables, such as the species and eating habits, the rate of feeding and the period of life of the animal (Costa-Bomfim *et al*., 2013; Sales *et al*., 2016; Zhang *et al*., 2021). Lack of knowledge of this variable can give rise to waste of food and financial resources.

Thus, success in cultivating a species depends on basic knowledge of both food management and feeding frequency. Thus, the objective of the present experiment was to adjust the feeding frequency to maximize the performance of juvenile cobias.

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Furthermore, this research is part of the project "Digestibility and zootechnical performance of beijupirá (*Rachycentron canadum*) with inclusion of seaweed meal in the rations" and was recorded and evaluated, under no. 23185.001462-2018-66, by Comissão de Ética no Uso de Animais – CEUA, do Instituto Federal de Educação, Ciência e Tecnologia do Espírito Santo.

This experiment was carried out at the Laboratory for Nutrition and Production of Aquatic Organisms (LANPOA), located on the Piúma campus of the Federal Institute of Espírito Santo, from May to June 2019.

Before transportation, the fish underwent a 48-hour fasting period. 1,100 juveniles with an

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average weight of 2.0g (±0.4) and an average length of 8.7cm (±0.5) were packed into a 400L isothermal transportation box filled with seawater. After the transportation, the fish were acclimatized to the local water temperature (28.4°C) in a 15,000 L acclimatization tank. The acclimatization period took approximately one week, so that any possible anomaly could be evaluated, and for the fish to recover from the stress of transportation.

During the experimental period, the fish were fed with extruded feed (48.3g.kg^{-1} crude protein; 13.33g.kg^{-1} ether extract; 11.8g.kg^{-1} moisture). The diet was formulated with the following ingredients: flattened rice (20.0g.kg^{-1}), poultry byproducts (20.0g.kg^{-1}), soy protein concentrate (16.94g.kg^{-1}), corn (15.5g.kg^{-1}), meat meal (10.0g.kg^{-1}), soy protein (6.1g.kg^{-1}), fish meal (5.0g.kg^{-1}), marine fish oil (4.46g.kg^{-1}), vitamin premix (1.0g.kg^{-1}) and mineral premix (1.0g.kg^{-1}).

Fish feeding was performed at different frequencies until apparent satiety was reached. The treatments were: T2 (feeding performed twice a day, at 06h and 20h), T4 (feeding performed four times a day, at 06h, 10h, 14h and 20h), T6 (feeding performed six times a day, at 06h, 08h, 12h, 14h, 18h and 20h) and T8 (feeding performed eight times a day, at 06h, 08h, 10h, 12h, 14h, 16h, 18h and 20h). Each treatment comprised three replicates.

The recirculating aquaculture system consisted of 12 circular polyethylene tanks (1,000 L each). The filtration system consisted of a mechanical/biological filter with a volume of 1,000 L containing gravel for solids retention; 0.2m^3 of biological media (mBBr 500m^2.m^{-3}) for fixing bacteria; an artificial aeration system; a protein fractionator (skimmer) with a flow rate of 5,000 L.h^{-1} and with a contact time of 90s for removal of organic solids; and two UV-C lamps (40W each) used for disinfection. The tanks were covered with a net to prevent accidental jumping deaths.

To maintain the water level, the water outlet from the fish tank was in the form of central drainage, and this outflow was directed to the mechanical/biological filter. By means of a 1.5HP pump, the water was returned to the experimental units through taps that allowed the water flow to each experimental unit to be controlled. In addition to supplying the experimental units, water was pumped into the UV filter and skimmer; this water was then returned to the mechanical/biological filter. The individual aeration system was driven by a radial compressor, and air was diffused in each tank through porous stones, 24 hours a day.

The marine water used in the experiment came from the natural environment, from Acaica Beach, located in Piúma approximately 100 meters from the laboratory. The maximum volume of each collection was 25,000 L; disinfection was performed using chlorine at 10 ppm. During the experiment, the salinity of the water in the system was controlled through use of fresh water from the public supply network, after removal of chlorine from this water by adding sodium thiosulfate. Marine water was kept in storage for use as replacement of losses caused by evaporation, biometrics, metabolic purposes, possible leakages and siphoning of the tanks, which was performed whenever necessary for removal of feces and leftovers of feed.

For the beginning of the experiment, all the fish were weighed on an analytical balance (±0.001g) and measured with the aid of a caliper (±0.01 mm), in terms of their total length and height. The experiment comprised four treatments with three replicates (12 experimental units) and each experimental unit contained 45 fish of approximately 2.0 g. The experimental units were distributed in a completely randomized design. The photoperiod was controlled for 12 hours of light. After every 10 days of feeding, a biometric evaluation was performed on all the fish, following the same initial procedure. Before each biometric evaluation, the fish remained fasting for approximately 15 h. The total duration of the experiment was 40 days.

The physicochemical water parameters of salinity, dissolved oxygen (mg.L^{-1}) temperature (ºC), total ammonia nitrogen (mg. L^{-1}) and pH were monitored and controlled daily. These parameters were measured at the time of the first feed of the day. Dissolved oxygen and temperature levels were measured using a portable digital oximeter (YSI 550a); pH with portable digital pHmeter (mPA210) and salinity with a refractometer. Total ammonia nitrogen and nitrite contents were analyzed by means of the LabconTest saltwater analysis kit.

To obtain survival values (S), weight gain (WG), specific growth rate (SGR) and feed conversion (FC), the following equations were used, respectively: number of fish at the end of the experimental period - number of fish at the start of the experimental period; final weight - initial weight; (ln(final weight)-ln(initial weight)/period)*100; feed intake/weight gain.

Mixed linear models were analyzed using the MIXED procedure of the Statistical Analysis System (SAS System, Inc., Cary, NC, USA) and, in cases of significant difference, the Tukey test was applied at 0.05 probability. Before the analyses using the MIXED procedure, the variables were evaluated for normality and homoscedasticity by means of the Shapiro Wilk and Breusch-Pagan tests, using the UNIVARIATE and ANALYTICAL SYSTEM MODEL (SAS) procedures. The survival estimate was calculated using a nonparametric estimation (Kaplan and Meier, 1958).

All results can be seen in Table 1. A significant difference ($P < 0.05$) was observed regarding weight at the end of the experimental period (40 days of experiment). The fish from treatment T2 presented significantly lower weight than the fish from treatment T8. No significant difference was observed ($P > 0.05$) regarding the variables of total length (cm) and height (cm). The same result was observed for weight gain (g).

Table 1. Variables (mean ± standard error) of cobia juveniles submitted to different feeding frequencies during the 40-day trial. Different letters in the same line represent a significant difference ($P < 0.05$) between treatments. (WG - weight gain; SGR - specific growth rate; FC - feed conversion ratio)

Variable	Time (days)	Treatment			
		T2	T4	T6	T8
Weight (g)	0	2.2600±1.6278	2.0933±1.6278	2.3800±1.6278	2.0267±1.6278
	10	7.7567±1.6278	9.000±1.6278	9.0667±1.6278	9.8201±1.6682
	20	17.0498±1.6692	19.7362±1.7146	20.0582±1.7689	20.5897±1.7145
	30	34.6577±1.7145	38.4750±1.7648	37.2166±1.7689	39.6436±1.7145
	40	56.7250±1.7648[b]	64.7209±1.8252[ab]	61.5539±1.7145[ab]	66.0051±1.7145[a]
Length (cm)	0	9.34±0.2717	9.27±0.2717	9.55±0.2717	9.38±0.2717
	10	12.45±0.2717	12.87±0.2717	12.99±0.2717	13.11±0.2717
	20	15.06±0.2717	15.88±0.2780	16.24±0.2779	16.20±0.2779
	30	18.68±0.2848	19.27±0.2848	19.27±0.2779	19.54±0.2779
	40	21.60±0.2848	22.15±0.2924	21.95±0.2779	22.15±0.2779
Height (cm)	0	0.8867±0.0313	0.8933±0.0313	0.8933±0.0313	0.8800±0.0313
	10	1.3400±0.0313	1.3933±0.0313	1.3467±0.0313	1.3733±0.0313
	20	1.4733±0.0313	1.4928±0.0313	1.4643±0.03218	1.500±0.0313
	30	1.8384±0.0331	1.9087±0.0331	1.8285±0.03218	1.9065±0.03218
	40	2.1922±0.0331	2.1750±0.0331	2.1857±0.03218	2.3208±0.03218
WG (g)	10	5.2667±1.4072	6.9067±1.4072	6.6867±1.4072	7.5467±1.4072
	20	9.2333±1.4072	10.1775±1.4072	10.2167±1.4072	10.4333±1.4072
	30	17.8500±1.4072	18.5941±1.4072	17.7189±1.4072	18.7200±1.4072
	40	23.6383±1.4072	25.3284±1.4072	24.9156±1.4072	25.6900±1.4072
SGR (%)	10	12.0364±0.5209[b]	14.5654±0.5209[ab]	13.3504±0.5209[ab]	15.5313±0.5209[a]
	20	7.9979±0.5209	7.6034±0.5209	7.5190±0.5209	7.3556±0.5209
	30	7.2494±0.5209	6.7716±0.5209	6.5551±0.5209	6.5923±0.5209
	40	5.2055±0.5209	5.1280±0.5209	5.1142±0.5209	5.0682±0.5209
FC	10	1.03±0.04	1.11±0.17	1.14±0.22	1.07±0.12
	20	1.22±0.10	1.19±0.12	1.36±0.35	1.42±0.34
	30	1.49±0.16	1.39±0.13	1.27±0.15	1.31±0.38
	40	1.47±0.14	1.51±0.35	1.46±0.09	1.31±0.09

For the variable of specific growth rate (%), a significant difference (P<0.05) was observed between the juveniles that received the T2 and T8 treatments over the first ten days of cultivation, such that the fish that received T8 presented a higher growth rate than the fish from T2.

The effect of feeding frequency is related to the size of the stomach of the species. Species with large stomachs require lower feeding frequencies to achieve maximum growth (Pillay and Kutty, 2005). Carnivorous fish, such as cobias, are morphologically able to ingest large prey as they manage to distend their stomachs to "tolerate" larger amounts of food. Thus, through a single feed, carnivorous fish are satisfied for a long period. On the other hand, herbivorous and omnivorous species have proportionally smaller stomachs, but larger intestines. This explains why species such as tilapia develop better with increasing feeding frequency (Riche *et al*., 2004; Sanches and Hayashi, 2001). Carnivorous species usually ingest foods with superior nutritional quality, while herbivorous species consume foods of low nutritional quality and therefore need to ingest a large amount of food to achieve their nutritional requirement. Thus, in addition to the morphology of the gastrointestinal tract, the quality of the di*et al*so influences the feeding frequency (Halver and Hardy, 2002).

In the present experiment, at the end of the experimental period, the juvenile cobias that received treatment with eight feedings per day presented higher weight than those that received two feedings per day. This result is different from that observed by Costa-Bonfim *et al*., (2013) among juveniles (100 to 200g) of the same species, who did not observe any differences regarding zootechnical performance variables. However, it needs to be noted that in the present experiment, the juveniles presented an approximate weight of only 2.0g. This difference in the stage of life of the fish between the experiments is probably the main reason for the difference in the results. In the literature, there is information that fish at earlier stages of development require higher feeding frequency (Liao *et al*., 2004; Nguyen *et al*., 2011). At the early stages of life, fish have a less developed gastrointestinal tract, in terms of both size and digestive capacity (Liu and Liao, 1999; Zhang *et al*., 2021). Corroborating this information, in the first ten days of the present experiment, the juveniles that received more feedings during the day had a better specific growth rate.

However, Sales *et al*. (2016) observed better rates of development for *Betta splendens* larvae fed twice a day, compared with larvae that received the treatment of four feedings per day. According to the authors themselves, high feeding frequency with smaller amounts of food on each occasion increases intraspecific competition, which increases the heterogeneity of the batch and decreases survival. Thus, the effect of feeding frequency would be directly related to the feed rate. Furthermore, Nguyen *et al*. (2011) found from working with cobia larvae that the highest feeding frequency possible is the most suitable regimen for the species, to minimize cases of cannibalism. In the present experiment, given that 2.0 g juveniles were used, the highest feeding frequency was also shown to be a more appropriate alternative. However, it is necessary to remember that in the present experiment, an *ad libitum* feeding regimen was used.

Since, in the present experiment, the feeding rate followed an *ad libitum* regimen, intraspecific competition for food was probably minimized. Therefore, survival in the present experiment may not have been affected by feeding frequency. In an experiment on juvenile cobias that were subjected to different feeding frequencies, Costa-Bonfim *et al*. (2013) did not observe any difference in survival. Similarly, Sanches and Hayashi (2001) and Sampaio *et al*. (2007) also did not observe any effect of food frequency on the survival of *Oreochromis niloticus* and *Odontesthes argentinensis*, respectively. For other species, such as *Astyanax bimaculatus* (Hayashi *et al*., 2004) and *Odontesthes humensis* (Pouey *et al*., 2012), different authors have demonstrated that food frequency can affect survival.

Thus, for juvenile cobias (2.0 g), it was observed that the feeding frequency of eight feeds per day, together with an *ad libitum* feeding rate, was suitable for improving the final weight of these fish, without causing problems like intraspecific competition or decreased survival.

Keywords: fish, marine, nutrient dynamics, saltwater, vertebrates

RESUMO

Para ajustar a frequência alimentar de juvenis de cobia ou beijupirá (Rachycentron canadum)*, 540 juvenis, com peso médio de 2,0 g (± 0,4) e comprimento médio de 8,7cm (± 0,5), foram distribuídos em quatro tratamentos: T2, T4, T6 e T8 (alimentação realizada duas, quatro, seis e oito vezes ao dia, respectivamente). Cada tratamento teve três repetições. Os juvenis de beijupirá foram alimentados em regime* ad libitum*. Observou-se diferença significativa no peso final (P<0,05), tendo os peixes do tratamento que receberam ração duas vezes ao dia (T2) apresentado peso significativamente menor do que os peixes que receberam oito refeições ao dia (T8). Não houve diferença nas variáveis conversão alimentar, ganho de peso, comprimento e sobrevivência (P>0,05) entre os tratamentos. Assim, para juvenis de beijupirá, observou-se que a frequência de alimentação de oito refeições por dia, juntamente com um regime de alimentação* ad libitum*, é a mais adequada durante essa fase. Os dados obtidos no presente experimento permitem otimizar uma etapa da cadeia produtiva do beijupirá.*

Palavras-chave: peixe, marinho, dinâmica de nutrientes, água salgada, vertebrados

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