http://dx.doi.org/10.1590/1678-4162-12543

***Arq. Bras. Med. Vet. Zootec.*, v.74, n.4, p.677-685, 2022**

Effect of different diet pellet sizes on the growth of juvenile cobia (*Rachycentron canadum*)

[*Efeito de diferentes tamanhos de* pellets *sobre o crescimento de juvenis de bijupirá (*Rachycentron canadum*)*]

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ABSTRACT

It is known that there is a close relationship between pellet feed size and fish growth. However, the magnitude of this relationship regarding an animals' feed efficiency is not yet clear. Therefore, the present study conducted at the Virginia Tech – Virginia Seafood Agricultural Research and Extension Center (USA), investigated the effects of three different pellet feed sizes, 1.7 mm (EP.1), 2.3 mm (EP.2) and 3.1 mm (EP.3), on the growth of Cobia (*Rachycentron canadum*) in a recirculating aquaculture system (RAS). Animals were farmed for 55 days in 77.5-liter tanks (6 fish with an initial density of 3.78 g L^{-1}) weighing approximately 41.83±1.24g. The results showed that during the 8-week culture period, there were no significant differences between the animal's final weight over the different pellet feed sizes (EP.1 - 952.5±40.7g; EP.2 - 1014.5±26.6g; EP.3 - 1030.0±54.8g). However, biomass gain showed significant differences (EP.1 - 704.0±34+.3g; EP.2 - 763.0±27.8g; EP.3 -776.5±51.9g). Consequently, significant differences were also found in the feed conversion factors.

Keywords: pellet size, feed, performance, *Rachycentron canadum*, growth

RESUMO

A relação entre o tamanho do pellet *da ração e o crescimento dos peixes é bem conhecida na literatura. No entanto, a magnitude dessa associação em relação à eficiência alimentar dos animais ainda não está clara. Portanto, o presente estudo conduzido na Universidade de Virginia Tech (Virginia Seafood Agricultural Research and Extension Center EUA), investigou os efeitos de três tamanhos diferentes de* pellets *da ração, 1,7 mm (EP.1), 2,3 mm (EP.2) e 3,1 mm (EP.3), sobre o crescimento de juvenis bijupirá* (Rachycentron canadum), *em um sistema fechado de recirculação. Os animais foram criados por 55 dias, em tanques de 77,5 litros (densidade inicial de 3,78 g / L), pesando 41,83 ± 1,24 g. Os resultados mostraram que, durante o período de cultivo equivalente a oito semanas, não houve diferenças significativas entre o peso final dos peixes para os diferentes tamanhos de* pellets *(EP.1 - 952,5 ± 40,7 g; EP.2 - 1014,5 ± 26,6 g; EP.3 - 1030,0 ± 54,8 g). No entanto, o ganho de biomassa mostrou diferenças significativas para o maior* pellet *quando comparado com o menor (EP.1 - 704,0±34+.3 g; EP.2 - 763,0±27,8 g; EP.3 -776,5±51,9 g). Consequentemente, diferenças significativas também foram encontradas nos fatores de conversão alimentar.*

Palavras-chave: tamanho do pellet, *alimentação, desempenho,* Rachycentron canadum, *crescimento*

INTRODUCTION

Feed is the dietary source in aquaculture and is normally administered as pellets in fish farms. Pellets contain the energy and nutrients that fish need to grow and develop healthily. There is a proportion between the energy in each food particle and its volume, in that the energy invested by fish to capture it cannot follow the same ratio for different particle sizes (Mattila and Koskela, 2018). Hence according to some authors, net energy gain may be very low for small particles and could result in low food conversion efficiency and slow growth (Jobling and Wandsvik, 1983; Santos *et al*., 1993). Furthermore, gastrointestinal research into fish has shown that many effects influence

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Submitted: September 23, 2021. Accepted: April 20, 2022.

evacuation time: temperature (Álvarez *et al*., 2010), nutrients (Adamidou *et al*., 2009) including small food particles under overload conditions that are more rapidly evacuated and can enter the intestine without sufficient gastric digestion to, thereby, reduce nutrient absorption efficiency (Jobling, 1986, 1988; Santos *et al*., 1993; Hossain *et al*., 2000).

However, another form of research indicates that feed particle size also strongly influences digestion and assimilation processes. It is known that smaller particles have a greater surface area, which improves the efficiency of digestive enzymes that contact the entire particle surface. Therefore, they are digested more rapidly or more completely than larger ones (Persson, 1986; Jobling, 1987; Hardy, 1989).

Thus, pellet size should be correlated to fish size so that the fish can grow as expected. It has been shown that the growth rate of fish is closely related to the pellet size of feed (Tabachek, 1988; Azaza *et al*., 2010). Food particle size has also been found to influence growth and food conversion in a wide series of fish species: Atlantic salmon *Salmo salar* (Wańkowski and Thorpe, 1979), sharptooth catfish *Clarias gariepinus* (Hossain *et al*., 2000), Arctic charr *Salvelinus alpinus* (Tabachek, 1988), common carp *Cyprinus carpio* (Hasan and Macintosh, 2008), gilthead sea bream *Sparus aurata* (Goldan *et al*., 1997) and Pike Perch *Sander lucioperca* (Mattila and Koskela, 2018).

Cobia, *Rachycentron canadum*, is considered one of the more promising candidates for warm-water marine fish aquaculture in the world (Liao *et al*., 2004; Benetti *et al*., 2010). In the past 10 years, attention has begun to focus on cobia as an aquaculture candidate given its fast growth and high feed efficiencies. Relatively little is known about cobia production in recirculating aquaculture systems (RAS). Therefore, the present study was designed to investigate the effects of three different pellet sizes on the growth of juvenile Cobia in RAS to determine the optimum food particle size.

MATERIAL AND METHODS

Fish and acclimation*:* The cobia in the study came from the brood stock fish at Trout Lodge Marine Farms (Vero Beach, Florida, USA), weighing 1g. They were fed commercial feed with 1.7mm size (Otohime Fish Diet - EP1, Marubeni Nisshin Feed, Tokyo, Japan) (Table 1). Acclimation lasted 55 days, until they reached the initial size utilized herein (the fish used in this experiment were never used in any prior experiments). The study was conducted at the Virginia Tech - Virginia Seafood Agricultural Research and Extension Center (VSAREC) (Hampton, VA, USA). And after acclimation 72 fish were stocked in 12 tanks randomly (6/tank) in RAS to initializing the trial.

Table 1. The Otohime feed, the commercial Fish Diet composition used on the effect of different pellet feed sizes on *Rachycentron canadum*

	EP1	EP2	EP3
Shape	Ext. Pellet	Ext. Pellet	Ext. Pellet
Size (mm)	1.7	2.3	3.1
Crude Protein %	48	48	48
Crude Fat %	14	14.5	14.5
Crude Fiber %	2	2	2
Crude Ash %	14	14	14
Crude Calcium %	2.2	2.2	2.2
Phosphorus %	1.7	1.7	1.7
Moisture %	6.5	6.5	6.5
Ingredients	Krill Meal, Fish Meal, Squid Meal, Wheat Flour, Potato Starch, Corn Starch, Tapioca Starch, Wheat Flour, Fish Oil, Calcium Phosphate, Astaxanthin, Calcium Carbonate, Betaine, Plant Gum Substance, Licorice Plant, Wheat Germ.	Krill Meal, Fish Meal, Squid Meal, Wheat Flour, Potato Starch, Corn Starch, Tapioca Starch, Wheat Flour, Fish Oil, Calcium Phosphate, Betaine, Plant Gum Substance, Licorice Plant, Wheat Germ.	Fish Meal, Wheat Flour, Tapioca Starch, Corn Starch, Potato Starch, Wheat Flour, Fish Oil, Calcium Phosphate, SoyWhey, Wheat Germ.

The nutritional composition of the feed offered is adequate for the species according to the scientific literature reviewed by Chou *et al*. (2001), Craig *et al*. (2006) and Fraser and Davies (2009).

Experimental design: The study consisted of three treatments: extruded pellet (EP.1; EP.2 and EP.3) corresponding to the different sized food pellets: 1.7 mm, 2.3mm and 3.1mm, respectively, with four repetitions totaling 12 tanks per trial. The pellet feed sizes were chosen for the trial according to the availability for the species, as stated by Benetti *et al*. (2010), Wills *et al*. (2013) and Chi *et al*. (2020), in studies with juvenile cobia (*Rachycentron canadum*). In each treatment, food particle size remained unchanged despite fish growth, so the particle size in relation to fish size diminished throughout the experiment. The RAS with 1860 liters consisted of twenty-four 77.5L tanks (but only 12 were used with fish) connected to a biofilter (988 L) with a 35% volume and plastic media-fixing bacteria (Kaldness, KMT, Norway), one mechanical filter (359 liters) (BubbleBead Filter, Aquatic Ecosystems, Apopka, FL, USA) and an ultraviolet 100 W (UV) light. The daily water exchange rates were 5.35% of the total system volume through two backwashes.

Experimental conditions: All the fish started with identical conditions, with weight and length of 41.83±1.24g and 20.0±0.51cm, respectively, which totaled a biomass of 251.2±7.5g per tank, in which six fish per tank were stocked (3.78g L^{-1}). Efforts were made to reduce the initial size heterogeneity within groups as much as possible and to minimize discrepancies among groups. The fish were slowly fed by hand to apparent satiation twice daily at 09:00h and 16:00h. Recirculation was interrupted while feeding took place in each tank to avoid food entering the return recirculation pipe, ensuring accurate food intake.

Water quality*:* All the water quality parameters were measured 3 times/week. Salinity, temperature, and dissolved oxygen (DO) were measured by YSI Pro-2030 (YSI, Yellow Springs, OH, USA). Salinity was maintained at 23.82±0.52mg L^{-1} (min 22.70mg L^{-1}, max 24.30mg L^{-1}) (Resley *et al*., 2006) by adding sea salt to the system. Temperature was set at 27.54±1.00ºC (min 23.2.0 ºC, max 28.20 ºC) (Sun and Chen, 2014) and was controlled by heat exchange and (DO) was maintained at 5.34±0.33 mg L^{-1} (min 4.68 mg L^{-1}, max 6.05 mg L^{-1}) by agitating the returned water and one supplemental air stone per tank. pH was measured by EcoSense pH100A (YSI, USA) and was maintained at 7.71±0.13 (min 7.34, max 8.00) throughout the trial. Alkalinity was measured daily to be adjusted between 120 mg L^{-1} and 160mg L^{-1} by adding sodium bicarbonate. The photoperiod was maintained at 13 h light/11 h darkness using fluorescent light. As well as water chemical quality, total ammonia nitrogen (TAN), nitrite and nitrate were assessed by a HACH DR 2800TM spectrophotometer (HACH Company, Loveland Colorado, USA), whose respective values were TAN (mg L^{-1}): 0.26±0.10 (min 0.05, max 0.51), NO_2 (mg L^{-1}): 0.16±0.23 (min 0.04, max 0.95) and NO_3 (mg L^{-1}): 15.23±4.16 (min 6.80 max 21.20).

Performance: The biometrics were run weekly throughout the trial. The fish from each tank were counted and collectively weighed (biomass) to determine biomass gain and survival. No food was distributed in the mornings on the initial weighing day and subsequent biometrics days. The parameters used to observe fish growth were biomass (w) during the trial, unit in grams; biomass gain (Δw); relative biomass gain (%w). The parameters employed to evaluate feed yield in the different pellet sizes: feed conversion ratio (FCR) and the specific growth rate (SGR). All the formulae for the parameters used are found in Table 2. In addition, the sigmoid growth curves (Boltzmann, Weibull, Richards, Gompertz, Logistic, and Morgan-Mercer-Flodin MMF) were fitted and compared by the sum of squared residuals and deviance.

Table 2. The formulae used to assess fish growth, absorption, and feed waste on trial with *Rachycentron canadum* testing the effect of different pellet feed sizes

Definition	Symbol	Unit	Formula	Description
Biomass gain	Δw	g	$\Delta w = w_1 - w_0$	w_0 = initial weight; w_1 = final weight.
Relative biomass gain	%w	%	$\%w = \frac{\Delta w}{w_0} x100$	---
Feed conversion rate	FCR	---	$FCR = \frac{f_t}{\Delta w}$	f_t = total amount of dry feed.
Specific growth rate	SGR	%	$SGR = \frac{\ln(w_1) - \ln(w_0)}{\Delta t} x100$	Δt = variation in time; ln = log base e.

Statistical analysis: Data were analyzed with the R software (R Core Team 2013), a language and environment for statistical computing (R Version 3.0.2). All the parameters were observed as normal distribution and homogeneity of variance by the Shapiro and the Bartlett test, respectively. Differences between treatments were compared by a one-way analysis of variance (ANOVA). Tukey's HSD post hoc test was used to compare among groups when differences were identified. The significance level was $p < 0.05$ in all the statistical tests. The tank mean values were considered observation units.

RESULTS

Growth performance and survival: The fitting of the growth data to a sigmoid curve provided the fit to each treatment: EP.1 – $y = (2917.31 + 1553.63x^{1.44}) / (11.80 + x^{1.44})$, $R^2 = 0.982$ and the sum of squared residuals (SSR) = 50752.51; EP.2 – $y = (5559.72 + 3190x^{1.15}) / (22.23 + x^{1.15})$, $R^2 = 0.997$ and SSR = 12262; and EP.3 – $y = (9211.37 + 4840.43x^{1.13}) / (36.58 + x^{1.13})$, $R^2 = 0.982$ with SSR = 76004. Where y = wet weight (g) and x = time (experimental week) (Fig. 1).

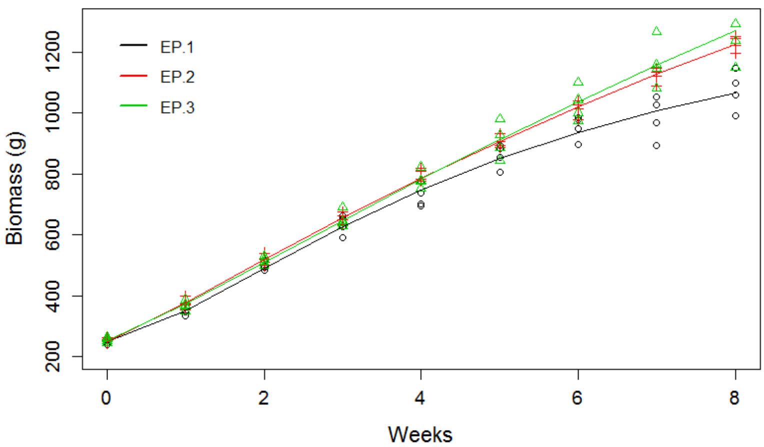

Figure 1. *Rachycentron canadum* biomass over time for each different pellet feed size.

No significant difference in biomass between tanks (p-value = 0.797; $p<0.05$) or per treatment (p-value = 0.564; $p < 0.05$) was found during the trial, such as the specific growth rate (SGR) (Table 3). However, significant differences were found, for the average biomass gain, between treatments EP.1 and EP.3 (p-value = 0.0116; $p < 0.05$) with a difference of 72.5g (9.34%) in average. The relative biomass gain (%w) showed a significant difference between treatment EP.1 and treatment EP.3 (p-value = 0.0314; $p<0.05$) (Table 3). No mortality occurred during the trial.

The letters after each value indicate the results of the pair-wise comparisons with significant differences ($p<0.05$). Data are represented (mean±SD) at the end of the experiment.

Table 3. The final biomass of *Rachycentron canadum* as the effect of different pellet feed sizes

Treatment	Mean biomass weight (g)	Biomass gain Δw (g)	Relative biomass gain %w	SGR
EP.1	952.5±40.7	704.0^{a}±34.3	268.7^{a}±13.1	3.20±0.047
EP.2	1014.5±26.6	763.0^{ab}±27.8	291.2^{ab}±10.6	3.32±0.100
EP.3	1030.0±54.8	776.5^{b}±51.9	296.4^{b}±19.8	3.33±0.115

Analysis of FCR: The weekly feeding rate between treatments was EP. 1 = 15.1%±7.7, EP. 2 = 13.5%±6.7, EP. 3 = 14.8%±7.2. The feed intake averages per tank were 122.34 g a week. As there was no strong evidence that the food ration in each tank differed (p-value = 0.29; $p < 0.05$), feed supply did not vary significantly per tank. The Boxplot shows a wide amplitude between treatments EP.3 and EP.2 (Fig. 2).

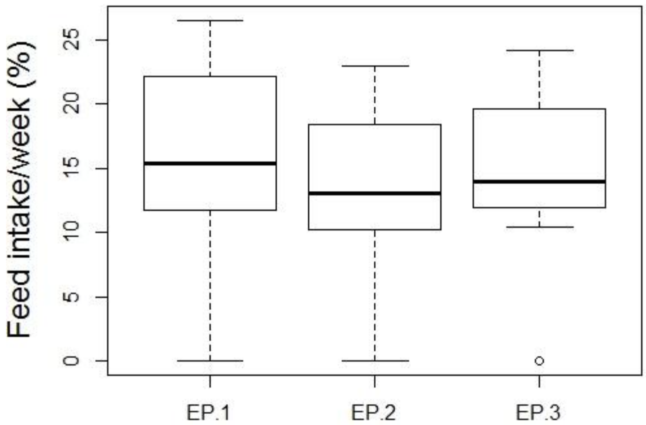

Figure 2. The Boxplot graph of the feed offered weekly for the *Rachycentron canadum* in each different pellet size trial

The FCR, decreased significantly with feed pellet size (EP.1 to EP.2) to 2.3 mm, and then increased and came close to EP.3 (Fig. 3). In addition, a significant difference was observed in the ANOVA test (p-value = 0.0085; $p < 0.05$). Therefore, the FCR for treatment EP.1 differed from treatment EP.2 (p-value = 0.0063; $p < 0.05$ by Tukey's Test) (Table 4).

The Fig. 4 shows the weight results of the weekly biomass for each treatment.

Table 4. Means and standard deviations of FCR among different pellets feed sizes fed to R*achycentron canadum*

Treatment	FCR	
	Mean	sd
EP.1 (1.7 mm)	1.27^{a}	±0.22
EP.2 (2.3 mm)	1.05^{b}	±0.09
EP.3 (3.1 mm)	1.32^{ab}	±0.34

sd - standard deviation. FCR – feed conversion.

DISCUSSION

One of the main variables that strongly influenced animal growth is the food supply. When fish were fed by hand until apparent satiety or for a given time, we were unable to totally control this variable precisely because it depends on the demand for fish. There was a difference in the satiety time of the feeds between different pellet sizes. During the trial, we observed that fish noted satiety faster with the 3.1 mm pellet size. This result reflects the variance in daily feed intake for this treatment (Fig. 1) when fish ate a lot in one day, and when they did not another day. Thus treatment EP.3 showed the widest variance (σ^2 = 216.17 g) in feed intake, treatment EP.1 was the second largest (σ^2 = 185.64 g) and EP.2 (σ^2 = 48.00 g) the third.

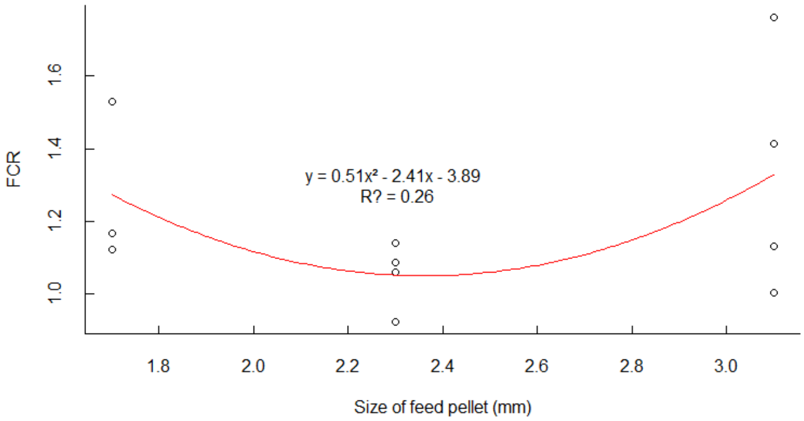

Figure 3. Regression of the feed conversion rate (FCR) in function on feed pellet size (mm) fed to Rachycentron canadum

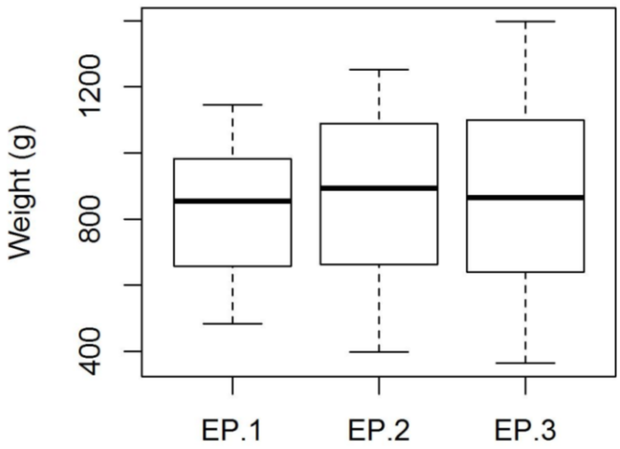

Figure 4. The Boxplot graph showing data distribution of weekly biomass (g) in different feed pellet size fed to Rachycentron canadum.

Fish gastrointestinal research has shown that the biggest food particles need more time to be evacuated (Jobling, 1986, 1988; Santos *et al.*, 1993; Hossain *et al.*, 2000; Adamidou *et al.*, 2009; Álvarez *et al.*, 2010), which can lead to a wide variation in the daily feed intake in each treatment.

The advantage of the apparent satisfaction method is that the feed offer estimation comes very close to the food intake estimation and this method, consequently, avoids food waste. When a feed time is stipulated, we expect fish to eat the same amount of food in all the pellet sizes but, in fact, fish spend more time watching small pellets and capturing them than larger pellets, and the opposite occurs with larger pellet sizes (Linner and Brännäs, 1994; Smith *et al.*, 1995). Thus, this methodology could possibly induce to offer a higher feed rate for the larger pellet sizes, than the smaller pellets sizes in the very long experiment. Furthermore, the handling of different feed sizes during a stipulated period can attenuate this outcome.

Overall, the fish in this study grew (on average) from 40 ±1.24g to a final overall average of 170 ±6.78g at 8 weeks. An expected result, when compared to other research, was carried out with juvenile cobia in both ponds and RAS (Chou *et al.*, 2001; Zhou *et al.*, 2005; Kenneth *et al.*, 2007). Biomass per tank was measured weekly and is plotted in Fig. 1. The best curve found was the sigmoid curve defined by Morgan-Mercer-

Flodin (MMF) using the sum of squared residuals as the decision criteria for choosing the best fit. The MMF model takes the expression below as the general equation:

$$f(t:\theta) = \frac{\beta\gamma + \alpha t^{\delta}}{\gamma + t^{\delta}};\ \theta = [\alpha, \beta, \gamma, \delta]$$

Morgan-Mercer-Flodin (MMF)

where $\alpha = 1553.64$; $\beta = 247.18$; $\gamma = 11.80$ and $\delta = 1.44$ for treatment EP.1, $\alpha = 3229.18$; $\beta = 249.52$; $\gamma = 22.43$ and $\delta = 1.15$ for treatment EP.2 and $\alpha = 4840.76$; $\beta = 251.83$; $\gamma = 36.58$ and $\delta = 1.13$ for EP.3. Very few studies have analyzed the Morgan-Mercer-Flodin (MMF) equation to model fish growth. Other curves were compared, such as Boltzman, Weibul, Richards, Gompertz and Logistic Model (the curves habitually used), but they all presented a higher sum of squared residuals compared to the MMF.

No significant difference was found in fish growth until 6 weeks of rearing. The biomass gain (Δw) and relative biomass gain (%w) in EP.3 were slightly higher than in both treatments EP.1 and EP.2 at the end of trial (table 3). Biomass variance increased with increasing pellet size, in such a way that the EP.1 weight was more homogeneous than for the other treatments (Fig. 4). While studying the effect of feed pellet size on the production parameters of Pike Perch (*Sander lucioperca*), Mattila and Koskela (2018) noted the same growth variation behavior according to increasing pellet size, by increasing the coefficient of variation in the final weight.

Growth heterogeneity can be explained by the evacuation time (Azaza *et al*., 2010), the energy spent on catching different pellet sizes (Matilla and Koskela, 2018), but also by the feed amount available for each individual per tank. The pellet number mean per one gram of feed in each treatment (EP.1, EP.2 and EP.3) was 371.00 nº/g±23.19; 103.78 nº/g± 3.99 and 61.78 nº/g±4.89, respectively. Thus, every meal in EP.1 allowed more pellets to be available per fish than other feed sizes, and avoided competition between individuals and sharing feed intake portions evenly. However, a bigger number of small pellets fed too quickly can cause waste by water leaching, because there is a limit to the number of pellets that can be captured and ingested caught by the fish in a time interval (Tabachek, 1988).

This study indicates that a different food size produces distinct FCR in Cobia. There is a specific pellet size per fish size, with a minimum point for FCR (Fig. 3). However, it is important to consider the small difference in commercial fish diet composition in its ingredients in this study. The same result has been found for Nile tilapia *O. niloticus* (Azaza *et al*., 2010) by quantifying the penalty (in terms of growth, food conversion and size heterogeneity) for feeding fish with particles below or above a peak. Tabachek (1988) has also observed the same result with *S. alpinus* data when studying the relationships among growth, fish size and particle size, and the relationships between growth and particle size when a single fish size was studied.

Although the FCR of treatment EP.2 showed no significant difference compared to EP.3, its values were better than EP.3 (Fig. 3), which is possibly because smaller particles have a bigger surface area, which improves the efficiency of digestive enzymes that contact the entire particle surface. Therefore, they are digested more completely than larger ones (Persson, 1986; Jobling, 1987; Hardy, 1989) and, thus, provide better feed efficiency. However, this cannot be observed for small pellets (EP.1); because the probability of them being seen and eaten by fish is very low, which would cause waste by water leaching and negatively affect feed efficiency. Thus, pellets sizes experiments may beget bias for small pellet in terms of Feed conversion rate (FCR).

The SGR was slightly below that found by Kenneth *et al*. (2007), but the initial weight and time interval in the present study were higher than in the above-cited study. So, using older individuals lowers the specific growth rate. However, the SGR were relatively satisfactory for this species sizes.

Therefore, applying an apparent satiety method in studies with different sized pellets to clearly show if there is a difference in feed supply between treatments is recommended as it can be an interference variable that is not considered in the experimental design.

CONCLUSION

Different pellet sizes provide distinct FCRs and feed efficiency in Cobia. A small pellet size up to 2.36 mm for fish weighing about 50 g grown in 8 weeks provides better feed efficiency. Therefore, lower pellet size values cause wastage by water leaching, which leads to poor feed efficiency, as well as very large pellets feed leading to high FCR values, which should be avoided. This study contributes to knowledge about feed pellet sizes in research, provides information for feed industries, suggests improvements for feed utilization on industrial farms and is in accordance with sustainability as feed waste lowers.

ACKNOWLEDGMENTS

Special thanks go to researchers and employees who participated in this study, the Laboratório de Piscicultura Marinha at the Universidade Federal Rural de Pernambuco, and the Virginia Tech Seafood AREC. This is a contribution of the Virginia Tech Seafood AREC (Agricultural Research and Extension Center).

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