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Effects of different sampling intervals on apparent protein and energy digestibility of common feed ingredients by juvenile oscar fish (*Astronotus ocellatus*)

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ABSTRACT. This study evaluated the apparent protein and energy digestibility of common feed ingredients (soybean meal, fish meal, wheat meal and corn) by juvenile oscars using two different sampling intervals (30 min. and 12h). The 160 juvenile oscar fish tested (22.37 ± 3.06 g BW) were divided into four cylindrical plastic net cages, each one placed in a 1000 L feeding tank. The experiment was completely randomized in a 2 x 4 factorial design (2 feces collection intervals and 4 feed ingredients) with four replications. The statistical tests did not detect an interaction effect of sampling interval and type of ingredient on digestibility coefficients. Sampling interval did not affect protein and energy digestibility. The physical characteristics of juvenile oscar feces likely make them less susceptible to nutrient loss by leaching and can therefore be collected at longer intervals. Protein digestibility of the different ingredients was similar, showing that apparent digestibility of both animal and plant ingredients by juvenile oscars was efficient. Energy digestibility coefficients of fish meal and soybean meal were higher than those of wheat meal and corn. Carbohydrate-rich ingredients (wheat meal and corn) had the worst energy digestibility coefficients and are therefore not used efficiently by juvenile oscars.

Keywords: cichlid, digestible energy, nutrition, digestible protein, fishes.

Diferentes intervalos de coleta na determinação da digestibilidade aparente da proteína e da energia de ingredientes comuns para o apaiari (*Astronotus ocellatus*)

RESUMO. O presente estudo avaliou a digestibilidade aparente da proteína e da energia de ingredientes (farelo de soja, farinha de peixe, farelo de trigo e milho) por juvenis de apaiari (*Astronotus ocellatus*) usando dois diferentes intervalos de coleta (30 min. e 12h). Os 160 juvenis de apaiari utilizados (22,37 ± 3,06 g de peso corporal) foram divididos em quatro tanques rede plásticos e cilíndricos, cada um colocado em um tanque de alimentação de 1.000 L. O experimento foi inteiramente casualizado em esquema fatorial 2 x 4 (2 intervalos de coleta de fezes e 4 ingredientes foram) com quatro repetições. Os testes estatísticos não detectaram efeito da interação entre o intervalo de coleta e tipo de ingrediente nos coeficientes de digestibilidade. O intervalo de coleta não afetou a digestibilidade da proteína e da energia. As características físicas das fezes dos juvenis de apaiari aparentemente as tornam menos sensíveis à perda de nutrientes por lixiviação, permitindo intervalos de coleta maiores. A digestibilidade da proteína dos ingredientes avaliados foi semelhante, mostrando que a digestibilidade aparente de ingredientes animais e vegetais por juvenis de apaiari é eficiente. Os coeficientes de digestibilidade da energia foram maiores para a farinha de peixe e o farelo de soja comparado a farelo de trigo e milho. Ingredientes ricos em carboidratos (farelo de trigo e milho) apresentaram os piores coeficientes de digestibilidade da energia e, portanto, não são usados eficientemente pelos juvenis de apaiari.

Palavras-chave: ciclídeos, energia digestível, nutrição, proteína digestível, peixes.

Introduction

Variations in the absorption of food ingredients by different species can be quantified by the digestibility coefficient, which measures the ability of the animal to retain nutrients and energy contained in a particular ingredient (NOSE, 1985; REIGH et al., 1990). In addition to providing support to diet formulations that meet the nutritional needs of animals, the evaluation of ingredient digestibility reduces nutrient losses.

Many devices and procedures (feces collectors, siphoning, stripping and dissection) are used to collect fish feces in digestibility studies (ABIMORAD; CARNEIRO, 2004). This collection can also be performed by decantation, and although this technique does not hurt or kill fish, it may cause

nutrient leaching from feces (VANDENBERG; DE-LA-NOÜE, 2001). Therefore, optimal feces collection intervals must be determined to avoid nutrient leaching (ABIMORAD; CARNEIRO, 2004).

The oscar fish (*Astronotus ocellatus*) is a large cichlid from the Amazon Basin. It is very popular as an ornamental fish, not only because of the wide range of colors and shapes, but also because it is a very intelligent fish that interacts with the hobbyist (PRONEK, 1982). Due to its delicious taste oscar meat is also exploited in Brazil for food. This fish species does not require high water quality conditions and reproduces in environments with low water renewal (SILVA, 2005).

Studies on oscar fish feeding habits are still scarce (SILVA, 2005) and ingredient digestibility is poorly known. The aim of the present study, therefore, was to evaluate energy and protein digestibility of feed ingredients (fish meal, soybean meal, corn and wheat meal) by juvenile oscars at different sampling intervals.

Material and methods

This study was conducted at the Aquaculture Center (CAUNESP) of the *Universidade Estadual Paulista* (UNESP), Jaboticabal, São Paulo State, from October 2007 to March 2008.

We used 160 juvenile oscars (22.37 ± 3.06 g BW), kept in four cylindrical plastic net cages, each one inside a 1000 L feeding tank. The tanks were supplied with an aeration system, with air stones connected to a central compressor and electric heaters with thermostats to maintain water temperature. The experiment was set in a completely randomized 2 x 4 factorial design, with 2 feces collection intervals (30 min. and 12h), 4 ingredients (soybean meal, fish meal, wheat meal and corn) and 4 replications (each consisting of one tank with 40 fish).

The partial excreta collection method and chromic oxide marker technique were used to determine protein and energy digestibility coefficients. Test diets contained 69% of a reference diet (Table 1), 30% of the ingredient tested (Table 2) and 1% of the marker. The reference diet was similar to that offered to juvenile oscars by Fabregat et al. (2006). The ingredients tested were ground in a hammer mill, mixed, moistened and pelleted in a meat grinder. The pellets were dried in a forced ventilation oven at 55°C for 24h and then fractionated and sieved to obtain a diameter of 0.71 mm.

Four glass-fiber collectors (200 L) (modified Guelph system) were manufactured to collect feces samples. Each feed tank was interconnected to a feces collector by a water recycling system. Water flow was maintained by air lift pumps and water was filtered by a foam filter. Water quality parameters (pH, dissolved oxygen, temperature and total ammonia) were monitored in both feed tanks and feces collectors after each feces collection. The tanks were cleaned every day to remove feces and food leftovers.

Table 1. Composition of the reference diet.

Ingredients (%)	
Soybean meal	26.60
Fish meal	24.10
Wheat meal	5.00
Corn	41.50
Soybean oil	1.3
Calcium phosphate	0.5
Vitamin and mineral supplement[1]	1.0
Composition	
Dry matter (%)[2]	90.00
Crude protein (%)[2]	32.05
Lipid (%)[2]	5.03
Gross energy (kcal kg^{-1})[2]	4,019.93
Crude fiber (%)[3]	3.55
Ash (%)[2]	8.87
Nitrogen-free extract (%)[3]	37.09
Calcium (%)[3]	1.82
Total phosphorus(%)[3]	1.26
Available phosphorus (%)[3]	1.03

[1]Vitamin mineral supplement (units kg^{-1} base diet): 5,000,000 IU vitamin A; 200,000 IU vitaminin D_3; 5,000 IU vitamin E; 1,000 mg vitamin K_3; 15,000 mg vitamin C; 4,000 mg vitamin B_{12}; 1,500 mg vitamin B_1; 1,500 mg vit. B_2; 1,500 mg vitamine B_6; 50 mg biotin; 500 mg folic acid; 4,000 mg pantothenic acid; 12.25 g B.H.T.; 40 g choline; 5,000 mg Fe; 500 mg Cu; 1,500 mg Mn; 10 mg Co; 50 mg I; 10 mg Se and 5,000 mg Zn; [2]Analyzed values (HORWITZ, 1997); [3] Calculated values (ROSTAGNO et al., 2005).

Table 2. Composition of diet ingredients.

Chemical composition	Unit	Ingredients			
		Fish meal	Soybean meal	Corn	Wheat meal
Dry matter[1]	%	92.09	89.91	88.53	89.00
Crude protein[1]	%	62.40	46.36	9.43	11.59
Gross energy[1]	kcal kg^{-1}	3,833.00	4,203.70	3,996.90	3,853.12
Lipid[1]	%	5.76	2.03	4.03	1.20
Crude fiber[1]	%	-	4.27	2.17	9.71
Nitrogen-free extract[1]	%	-	27.81	71.62	75.20
Ash[1]	%	26.76	6.13	1.28	0.54
Calcium[2]	%	6.60	0.30	0.03	0.14
Total phosphorus[2]	%	3.50	0.65	0.24	0.05
Available phosphorus[2]	%	3.50	0.20	0.08	0.02

[1]Analyzed values (HORWITZ, 1997); [2]Calculated values (ROSTAGNO et al., 2005).

Fish were fed a commercial diet for 15 days to allow them to adapt to the experimental conditions. Thereafter, experimental diets were provided to satiation twice a day for 5 days. Ingestion of the different diets was similar. Fish were transferred to the collectors immediately after feeding on the morning of the sixth experimental day. Feces were collected at 30-minute intervals and stored in freezer. This procedure continued until the amount required to determine marker, crude protein and

gross energy levels was obtained. Feces collection was interrupted at the end of the day and the fish remained in the collector for an additional 12h. Feces were then collected only once, since the amount obtained was sufficient for laboratory analyses.

Water quality parameters over the experiment (mean ± sd) were: pH 7.24 ± 0.11, dissolved oxygen 5.70 ± 1.09 mg L^{-1}, temperature 30.43 ± 0.70°C and total ammonia 160.76 ± 58.41 mg L^{-1}. These values remained constant and were suitable for oscar fish cultivation (GURGEL, 1970).

The apparent protein and energy digestibility coefficient (ADC) of both reference and test diets was calculated based on quantitative analysis of markers and nutrients found in the diets and feces, using the following formula (NOSE, 1985):

$$ADC = 100 - \left[100 \left(\frac{\%\text{diet marker}}{\%\text{feces marker}} \text{x} \frac{\%\text{feces nutrients}}{\%\text{diet nutrients}} \right) \right]$$

The apparent protein and energy digestibility coefficient of each ingredient (ADC_i) was calculated using the equation described by Reigh et al. (1990):

$$ADCi(\%) = \left(\frac{100}{30} \right) \text{x} \left[\text{test diet} - \left(\frac{69}{100} \text{x reference diet} \right) \right]$$

where:

test diet = apparent digestible energy and protein content in the test diet;

reference diet = apparent digestible energy and protein content in the reference diet.

Statistical analysis was performed using Statistical Analysis System 8.0 software. Data were checked for homoscedasticity and normality before undergoing analysis of variance. The model evaluated the effects of the ingredient (I), sampling interval (IC) and IxIC interaction on digestibility coefficients. Significant statistical differences were compared by the Tukey test. Significance level was set at 5%.

Results and discussion

Protein and energy digestibility coefficients of the ingredients for juvenile oscar fish (Table 3) were not affected by the interaction between the factors studied (ingredient type and feces collection interval; $p > 0.05$).

Findings of a recent study (ABIMORAD; CARNEIRO, 2004) on juvenile pacu fish differ from those of the present study. However, these results cannot be considered contradictory because oscar and pacu are different species, with distinct metabolism and digestive physiology. Owing to their physical characteristics, juvenile oscar feces might be less susceptible to nutrient loss by leaching, allowing feces to be collected at longer intervals. Indeed, leach-resistant whole pellets can be collected from more consistent feces (CHO et al., 1985). Feces from other cichlids have good resistance against leaching and studies on the Nile tilapia provided consistent results using a 12h fecal collection interval (FURUYA et al., 2001; PEZZATO et al., 2002). To the best of our knowledge, specific studies on the adequacy of fecal collection intervals used in digestibility assays for fish are nonexistent. However, satisfactory results are generally obtained using 12h intervals, as evidenced in a number of studies (BOSCOLO et al., 2002; CHENG; HARDY, 2002; FURUYA et al., 2001; MEURER et al., 2003; PEZZATO et al., 2002; TIBBETTS et al., 2004; VANDENBERG; DE-LA-NOÜE, 2001).

Table 3. Crude protein and gross energy digestibility coefficients (F values; means ± sd) of juvenile oscar diet ingredients.

	F values	
	Digestibility coefficients for crude protein	Digestibility coefficients for gross energy
Sampling interval (S)	3.90 ns	1.54 ns
Ingredient (I)	1.35 ns	8.96**
Interaction (S x I)	0.40 ns	0.18 ns
CV (%)	7.30	16.70
Mean values for S (%±SD):		
30 min.	92.58 ± 3.17	76.61 ± 12.36
12 hours	90.03 ± 5.12	74.71 ± 16.42
Mean value for I (%±SD):		
Soybean meal	93.68±1.91	80.12±4.17a
Fish meal	92.82±4.44	86.77±5.17a
Corn	90.21±4.79	68.35±5.17b
Wheat meal	87.55±5.69	56.62±12.26b

Means followed by a same letter in a column do not differ by the Tukey test ($p > 0.05$)
** = Significant at $p < 0.05$; NS = Non significant

No information on ingredient digestibility for juvenile oscars is available in the literature. We found that the feed ingredients evaluated have similar protein digestibility for oscar fish ($p > 0.05$; Table 3). The protein digestibility coefficients obtained were higher than those observed for other omnivorous fish (ABIMORAD; CARNEIRO, 2004; FERNANDES et al., 2004; KÖPRÜCÜ et al., 2004; OLIVEIRA-FILHO; FRACALOSSI, 2006; SKLAN et al., 2004).

Protein digestibility was not related to type of ingredient. This indicates that juvenile oscars have efficient protein uptake from both animal and plant sources. A similar conclusion was arrived at by Fabregat et al. (2006), who studied different protein sources in juvenile oscar diets. They found that total

replacement of fish meal by soybean meal in diets did not affect feed efficiency although it compromised food consumption and fish performance.

Energy digestibility, in turn, was different among the evaluated ingredients ($p < 0.05$). The energy digestibility coefficients of fish meal and soybean meal were higher ($p < 0.05$) than those of corn and wheat meal, which are carbohydrate-rich ingredients. Carnivorous fish use protein and fat efficiently, but not carbohydrates because they lack the enzymes needed to digest this material (HIDALGO et al., 1999). Despite evidence that intestinal bacteria of oscars can digest proteins, carbohydrates and fats (RAMIREZ; DIXON, 2003), the present study indicates that nutrient use by juvenile oscars is similar to that of carnivorous fish. Other cichlids such as the Nile tilapia also do not use dietary carbohydrates efficiently (SHIAU, 2002).

A study on the digestive enzymes of 11 teleost fish showed that these enzymes are not related to type of diet or fish feeding behavior (CHAKRABARTI et al., 1995). According to the authors, fish can occupy more than one ecological niche because most of the species are opportunistic. Moreover, the food source varies according to its availability in the environment. The feeding habits of oscars range from omnivorous to carnivorous and their ecological niche is still a matter of discussion (SILVA, 2005). Considering that fish are opportunistic and that in the Amazon basin (the natural habitat of oscars) food availability varies greatly throughout the year, evaluation of stomach contents can provide misleading results on its feeding behavior. Therefore, this can be better assessed by investigating the digestive strategy for nutrient utilization (CHAKRABARTI et al., 1995).

Conclusion

The 30 min. and 12h intervals used to collect juvenile oscar feces did not affect protein and energy digestibility of feed ingredients. Apparent protein and energy digestion of both animal and plant feed sources by these fish was efficient. Carbohydrate-rich ingredients have the worst energy digestibility coefficients, showing that juvenile oscars do not use carbohydrates efficiently.

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