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Testing various faeces-collecting methods to improve digestibility studies with tambaqui, *Colossoma macropomum* (Cuvier, 1816)

By C. S. da Mota[1], J. G. Araújo[2], D. M. C. Pádua[3], G. P. Martins[2] and I. G. Guimarães[2]

[1]*Nutroeste Rações Nutrição Animal LTDA, Goiânia-GO, Brazil;* [2]*Laboratório de Pesquisa em Aquicultura, Universidade Federal de Goiás – Campus Jataí, Jataí-GO, Brazil;* [3]*Laboratório de Pesquisa em Organismos Aquáticos, Goiânia-GO, Brazil*

Summary

Determining the ingredients and/or digestibility of a diet is one of the most important steps in fish nutrition for accurate diet formulation. Additionally, the use of digestible nutrient data in fish feed formulation can reduce the environmental impact of aquaculture. To properly determine the nutrient digestibility (ADC) of a feed ingredient and/or compound diets, an accurate faeces-collecting method should be used to provide reliable information on the digestion capacity of a determined species. Thus, a series of two trials were performed aiming to evaluate and standardize a faeces-collecting method for *Colossoma macropomum*. Faeces from all groups were collected using one of the methods. In trial 1, two faeces-collecting methods (sedimentation and dissection) were evaluated using 80 juveniles (170 g average weight) randomly assigned into four 310-L aquaria. In trial 2, 135 juveniles (300 g average weight) were randomly distributed into nine 310-L aquaria and fed a control diet (without binder) and diets containing a dietary binder at 0, 2 and 4 g kg^{-1} diet aiming to test the effect of binder inclusion on the ADC of nutrients. Fish were fed in triplicate groups per diet. Results showed that the dissection method provided the lowest ADC values for dry matter, phosphorus and gross energy. ADC of dry matter tended to decrease up to 5 h (305 min) of faeces immersion and seem to stabilize afterwards. Using 4 g kg^{-1} of dietary binder tended to reduce the leaching of DM, and no effect was observed on the protein digestibility. Phosphorus was the nutrient most prone to leaching in faeces. Faeces collection within 1 h is recommended in the sedimentation method in *C. macropomum* digestibility studies; the use of dietary binder at 4 g kg^{-1} may be a useful approach to reduce nutrient leaching in the faeces.

Introduction

The aquatic environment is challenging for digestibility studies in fish due the difficulty of sampling faeces from the water, the accurate measurement of feed intake and the contamination of faeces with ingested feed. The direct method of collection needs an accurate determination of feed intake and total faeces recovery, which is rarely used in digestibility studies in fish. Among the direct methods reported, we could identify the metabolic chamber (Halver, 1989) and continuous filtration adapted to total faeces collection (Choubert et al., 1982). However, due to the high stress to which fish are submitted using these methods, they have not been used extensively. Besides, the indirect method of faeces collection estimates the digestibility based on the difference between the concentration of an indigestible marker on faeces and feed. In this method, faeces can be collected: (a) directly in the water after defecation; or (b) from the posterior section of fish intestine before defecation (Portz, 2001). Several authors have postulated that the faeces collecting methods performed out of the water, such as dissection (Henken et al., 1985), stripping (Vens-Cappell, 1985) or anal suction (Brown, 1993; are more adequate than other methods due to reduced leaching of nutrients in the water. However, these methods require constant handling of fish, leading to an increased stress. Additionally, these methods tend to underestimate digestibility values as a result of expulsion of partially digested material or contamination with endogenous material (mainly the protein fraction) (Allan et al., 1999). Methods based on faeces collection in the water, such as faeces pipetting (Alliot et al., 1979), sedimentation in the water column (Cho et al., 1985) or continuous mechanical filtration of effluent water (Choubert et al., 1982) reduce fish stress, but tend to increase nutrient leaching in faeces (De La Noüe and Choubert, 1986), except for the continuous filtration method. Thus, each method has advantages and drawbacks, particularly if we consider the easy way of collecting samples and the nature of recovered faeces. However, collection of representative volumes of faeces in fish weighing less than 1 kg is a problem in both methods (Windell et al., 1978; Ferraris et al., 1986; Glencross et al., 2007; Blyth et al., 2015).

A nutritional approach that can reduce the nutrient leaching in faeces is the inclusion of some specific dietary binders at low levels into fish feeds (Brinker et al., 2005). Although the dietary binders have been used extensively and investigated when fish were fed pelleted diets, once the use of extruded diets became more widespread, few publications addressed the potential of using these additives. However, the available literature have demonstrated a promising research venue for the beneficial use of some dietary binders (e.g. gum guar and alginate) in fish diets, especially in salmonids, for improving the physical characteristics of faecal pellets and thus reducing nutrient and dry matter leaching (Brinker et al., 2005; Brinker, 2007, 2009). Furthermore, to

the best of our knowledge, just one study was aimed at using this nutritional approach as a means to standardize a faeces collecting method for digestibility studies in fish (Cantelmo et al., 1999).

Despite the economical and social importance of *Colossoma macropomum* for South America, few studies have properly evaluated the effect of faeces collecting methods on apparent digestibility determination (Silva et al., 1999) and a description of the physical characteristics of the faeces of this species. Additionally, this information is of utmost importance since the future of *C. macropomum* farming depends on the production of more efficient and specific diets to reduce production costs and be environmentally sustainable. Thus, we designed a series of trials to evaluate the feasibility of using different faeces collecting methods for *C. macropomum* and whether the use of low inclusion levels of feed binders could alleviate leaching to improve collecting methods and the reliability of faeces content analyses.

Materials and methods

A previous trial (data not shown) was undertaken to measure nutrient leaching in *C. macropomum* faeces and compare the commonly used faeces collecting methods used in digestibility studies in fish (stripping, modified Guelph system and dissection). Based on those results, we designed Trial 1 to compare the feasibility of using a dissection method or a sedimentation method previously reported for Nile tilapia (Guimarães et al., 2008a) and many other species. Although we tried to include the stripping method in the evaluation, an insufficient faeces volume was collected before fish began to die after 3 weeks of collection had commenced, even when using a high number of fish (50 fish per group). Based on the results of Trial 1, we designed a second trial (Trial 2) to evaluate whether the supplementation with a dietary commercial binder used in aquaculture could reduce the nutrient leaching of faeces in the water and its effects on the concentration of nutrients in different sections of the intestinal tract.

Experimental diets and diet preparation

In Trial 1, a basal diet (Table 1) was formulated to meet the protein and energy requirements for *C. macropomum* (Oishi et al., 2010). Chromic oxide (Cr_2O_3) was used as an external inert marker at 2.0 g kg^{-1} according to Bremer Neto et al. (2005). Ingredients were supplied by Mogiana alimentos S.A (Anapolis, Brazil). This diet has been successfully used in our laboratory without compromising fish health or growth (Santos, 2012). The diets from Trial 2 (Table 2) were basically the same used in the first experiment with slightly modifications, to which a commercial dietary binder (NutriBinder®, Venture Minerals & Resources Inc, Lafayette, USA) was included at 2.0 and 4.0 g kg^{-1}. NutriBinder® is a mixture of modified carbohydrates derived from sorghum used in shrimp feeds to increase pellet stability. All ingredients were ground and sieved to 500 μm. Diets were mechanically mixed with water (30% of dry-weight) in a Kitchen Aid multi-function mixer and the moist mixture extruded at 65–78°C in a meat grinder and exited in a 4.0 mm die. Diets were oven-dried at 55°C for 48 h, crumbled to obtain 3 cm long pellets, and stored at −18°C until used.

Fish and experimental condition – Trial 1

For the sedimentation method, 120 juveniles (170 ± 8.17 g) were randomly stocked into four 310-L aquaria and kept in a recirculating water system with automatically controlled water quality parameters (temperature, dissolved oxygen (DO), ammonia and pH). Four conic 300-L aquaria were used to collect faeces by sedimentation following a modified procedure of the Guelph system described previously (Guimarães et al., 2008a,b). Faeces collection was performed according to Guimarães et al. (2008a) with slight modifications. Briefly, fish were fed 7 days prior to the beginning of faecal collection (acclimatization period). Fish were fed three times daily until apparent satiation. Groups of fish were then transferred to collecting (sedimentation) aquaria to start the scheduled faeces removal in fixed time intervals. In order to evaluate whether the moment of faeces sampling had an effect on digestibility, four sampling times were tested in each group: (T1) 60 min after the drop of the first faecal pellet occurred (0–60 min); (T2) = 120 min (0–120 min); (T3) = 240 min (0–240 min) and (T4) = 480 min thereafter (0–480 min). Fish were then returned to the feeding aquaria. This procedure was repeated every other day until a representative volume of faeces could be used for further analysis. We used the ADC of nutrients as an indirect method to assess nutrient leaching in faeces. Faeces were oven-dried at 55°C, ground and stored at −20.0°C until chemical analysis.

The same groups of fish were used for the dissection faeces collection method 7 days after the end of the faeces collection by the sedimentation method to ensure the same adaptation period in both methods. For this purpose, fish were euthanized with an overdose of anaesthetic (Eugenol, Vetec Industry) and their intestinal tracts dissected carefully to

Table 1
Composition of experimental diet, Trial 1[a]

Ingredients	g kg^{-1}
Soybean meal	490
Fishmeal	50
Corn	270
Wheat middlings	60
DL-methionine	1
Cr2O3	1
Soybean oil	65
Dicalcium phosphate	56
Vitamin C	0.8
NaCl	1
Vitam/min. mix[b]	5
BHT[c]	0.2
Total	1000

[a]280 crude protein g kg^{-1}, 9.4 g kg^{-1} total P, 927 g kg^{-1} dry matter and 18 Mj kg^{-1} gross energy.
[b]Vitamin mixture, per kg $diet^{-1}$: vitamin A, 16060 IU; vit. D3, 4510 IU; vit. E, 250 IU; vit. K, 30 mg; vit. B1, 32 mg; vit. B2, 32 mg; calcium pantothenate, 80 mg; niacin, 170 mg; biotin, 10 mg; folic acid, 10 mg; vit. B12, 32 μg; vit. B6, 32 mg.
[c]Antioxidant Butylhydroxytoluene.

Table 2
Composition of experimental diets, Trial 2 (g kg^{-1})[a]

	Binder level		
Ingredients	0	2	4
Soybean meal	406	406	406
Corn gluten meal	60	60	60
Fishmeal	50	50	50
Corn	270	270	270
Wheat middlings	68	68	68
Binder	-	2	4
Lysine HCl	2	2	2
DL-methionine	1	1	1
Cr2O3	2	2	2
Starch	4	2	0
Soybean oil	70	70	70
Dicalcium phosphate	60	60	60
Vitamin C	0.8	0.8	0.8
NaCl	1	1	1
Vit./min. mix[b]	5	5	5
BHT[c]	0.2	0.2	0.2
Total	1000	1000	1000

[a]280 crude protein g kg^{-1}, 9.4 g kg^{-1} total P, 927 g kg^{-1} dry matter and 18 Mj kg^{-1} gross energy.
[b]Vitamin mixture, per kg $diet^{-1}$: vitamin A, 16060 IU; vit. D3, 4510 IU; vit. E, 250 IU; vit. K, 30 mg; vit. B1, 32 mg; vit. B2, 32 mg; calcium pantothenate, 80 mg; niacin, 170 mg; biotin, 10 mg; folic acid, 10 mg; vit. B12, 32 μg; vit. B6, 32 mg.
[c]Antioxidant Butylhydroxytoluene.

avoid contamination with other fluids and tearing the tract in unspecified regions. Intestinal tract was equally divided in three different sections after the pyloric caeca (anterior or proximal, medium and posterior sections). A representative volume of faeces was collected from each intestinal section, then frozen and stored at −20.0°C until further chemical analysis. For the dissection method, fish were euthanized after 2 h of being fed because in a previous trial we observed that it took approximately 2 h for faeces to be found in the posterior section of the intestinal tract.

Fish and experimental condition – Trial 2

In this study, 135 juveniles (300 ± 10.08 g) were randomly stocked into nine 310-L aquaria connected to a recirculating system. Five conic 300-L aquaria were used to collect faeces by sedimentation. Three diets containing graded levels of dietary binder (Table 2) were randomly assigned to aquaria and fish were fed 7 days prior to the beginning of faecal collection (acclimatization period). For faeces collection, the first five groups of fish were transferred to a conical aquaria; the remaining four groups were transferred on the consecutive day. This procedure was carried out until a representative volume of faeces per replicate was collected.

Procedure for faeces collection by the dissection method was the same as in Trial 1.

Temperature and dissolved oxygen (DO) were measured each day in all tanks throughout the trial; pH and total ammonia-nitrogen were measured every other day in four randomly chosen tanks, except for the sedimentation aquaria where measurements were taken in all tanks. During the experimental period of both trials, a 12 h light : 12 h dark photoperiod was maintained, DO content was 6.3 ± 0.9 mg L^{-1}, pH 7.2 ± 0.6, and total ammonia–nitrogen 0.048 ± 0.021 mg L^{-1}. Temperature was 29.7 ± 1.2°C.

Chemical Analysis

Chemical analyses of diets and faeces were determined according to AOAC (1990) protocols. Chromic oxide content of diets and faeces were determined according to Bremer Neto et al. (2005) and gross energy content was determined in an adiabatic calorimetric bomb (Parr Instrument Company, Moline, IL, USA).

ADC Calculation

Apparent digestibility coefficient (ADC) was calculated according to the equation described by Cho et al. (1985):

$$\mathrm{ADC}\,(n) = 100 - \left[100\left(\frac{\%\mathrm{Cr_2O_3d}}{\%\mathrm{Cr_2O_3f}}\right) \times \left(\frac{\%\mathrm{Nf}}{\%\mathrm{Nd}}\right)\right]$$

where ADC (n) = apparent digestibility coefficient of a nutrient (n) in the diet; Cr_2O_3d = % chromic oxide of the diet; Cr_2O_3f = % chromic oxide of the faeces; Nd = nutrients in the diet; Nf = nutrients in the faeces.

Statistical Analysis and Experimental Design

All percentage data were arcsin-transformed and verified for normality (Kolmogorov–Smirnov test) and homogeneity of variances (Levene's F test). The experimental design used in Trial 1 was completely randomized with five treatments (a faeces collecting method and a sedimentation collecting method at four different periods) and four replicates (groups of fish). These results were subjected to one-way ANOVA; significant differences on ADCs among treatments were determined using a SNK multiple range test. Regression analysis was used to analyze the relationship between ADC of nutrients and collection periods only in the sedimentation method assay. Models were chosen based on r^2 value and the least sum of squared differences between the values of the observed and predicted values of the dependent variable (Shearer, 2000). To test whether the inclusion of dietary binder affected ADC values in each faeces-collecting method in Trial 2, we analyzed the data separately. To evaluate the differences in ADC regarding the dietary binder and the intestinal section, a completely randomized design following 3 × 3 factorial arrangement with three replicates was used, while a 2 × 3 factorial arrangement with three replicates was used to evaluate the effects of the faeces collection method and dietary binder. Data were submitted to a two-way ANOVA; in the interaction between the factors, Scheffé *post-hoc* test or F test was used to compare the treatment means.

Results

The faeces collecting method and moment of faeces sampling affected nutrient digestibility coefficients in *C. macropomum*

($P < 0.05$). We observed a linear increase of ADC of P with the duration of faeces in the water column, while a quadratic relationship was observed between the length of faeces in the water column and the ADC GE (Fig. 1b). The dissection method showed the lowest ADC DM values, but was not significantly different from faeces collected after 480 min in the sedimentation method. A broken line model better described the relationship between the duration of faeces in the water and the ADC DM. After 305 min the ADC DM stabilized and no significant changes in ADC values were observed (Fig. 1a). The duration of faeces in the water did not affect the ADC CP. By comparing the two faeces-collecting methods at 60 min, we observed a significant increase in ADC DM and GE using the sedimentation method. The choice of using only the 60 min sampling period to compare with the dissection method was due to the continuous leaching of dry matter in the sedimentation method, thus we tried to standardize the conditions by using only the 60 min sampling. However, a significant decrease on ADC P was observed when faeces were collected from the water at 60, 120 and 240 min using the sedimentation method compared to the dissection method. ADC CP were significantly different between the dissection and the sedimentation collecting methods at 120 and 480 min (Table 3).

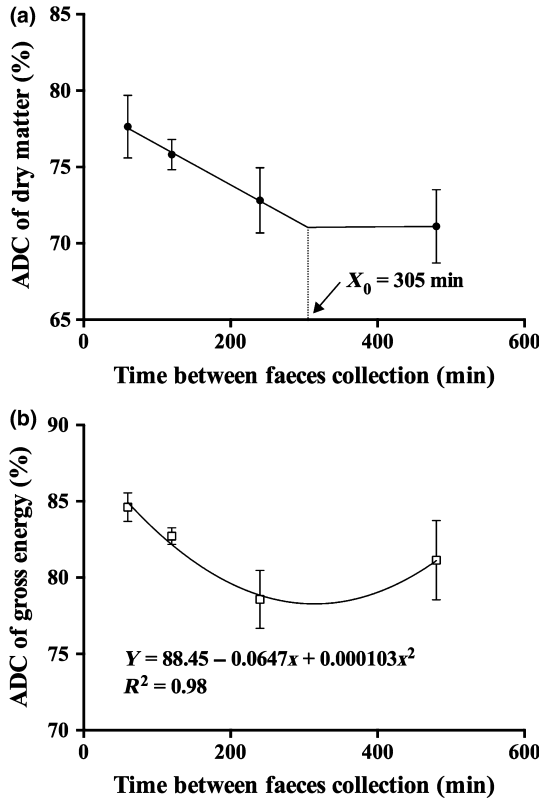

Fig. 1. Effect of time between faeces collection (60, 120, 240 and 480 minutes) on apparent digestibility coefficients (ADC) of dry matter (a) and gross energy (b) for *Colossoma macropomum*. Each point = average (±SD) of three groups of fish with faeces pooled from nine consecutive samplings

Binder inclusion and gut section significantly affected the ADC of DM, P and CP ($P < 0.001$) when faeces were collected by the dissection method (Table 4). Interaction between both factors was observed for ADC P and CP. In the dissection method, higher ADCs of DM, CP and P were observed when binder was included at 4 g kg^{-1} (Fig. 2). However, no differences were observed in the sedimentation method irrespective of the dietary binder level ($P > 0.05$). Regardless the dietary inclusion of binder, the ADC of nutrients calculated using faeces collected at the distal section of *C. macropomum* gut tended to be higher than in the other two sections, except for the ADC CP. Differences between the faeces collecting methods were observed for ADC of DM and CP when fish were fed diets containing 0 and 2 g kg^{-1} binder. However, no differences between methods were observed at 4 g kg^{-1} binder level. The ADC of P from faeces collected by the sedimentation method was significantly lower than when collected by the dissection method in fish fed diets containing 2 and 4 g kg^{-1} binder. Except for the ADC of P, no differences were observed between the methods when binder was included in the diets at 4 g kg^{-1} ($P > 0.05$) (Fig. 2).

The increase in binder concentration tended to decrease the variability and concentration of chromium quantification in faeces collected by sedimentation (Fig. 3a), while the highest dietary binder level significantly increased the chromium concentration in faeces collected at the distal section of *C. macropomum* gut ($P < 0.01$). Phosphorus concentration did not change significantly among the gut sections irrespective of dietary binder inclusion (Fig. 3b). However, faeces collected in the water column presented the highest phosphorus concentration. Neither the binder nor the faeces collection method affected protein concentration in *C. macropomum* faeces ($P > 0.05$).

Discussion

Although digestibility studies are important for the development of fish diets, relatively few studies are available on the effects of the faeces-collecting method on ADC of nutrients in fish (Glencross et al., 2007; Blyth et al., 2015) considering the great feeding plasticity and the associated changes in the morphology of the fish digestive tract. Additionally, main findings on the effects of faeces-collecting methods were obtained for salmonid fish (NRC, 2011), which greatly differs from some tropical fish species, such as tilapia (*Oreochromis niloticus*), *C. macropomum* or pacu (*Piaractus mesopotamicus*). Concurrently, the stripping method of faeces collection has been generally accepted as the more reliable method for assessing nutrient digestibility in fish (NRC, 2011; Blyth et al., 2015) due to the conservative, less variable ADC values produced using this method. However, each method presents positive features and drawbacks, and one method cannot always be applied to all fish species. In this regard, we conducted two series of studies to evaluate three commonly known faeces collecting methods in one trial; based on these results we tried to standardize one feasible, rapid and efficient method for *C. macropomum*, an economically important Amazonian species for most Latin American countries.

Table 3
Apparent digestibility coefficients of nutrients calculated using *Colossoma macropomum* faeces collected by sedimentation and dissection (n = 4, mean ± SD)

Collecting method	ADC_{DM}[2] (%)	ADC_{CP}[3] (%)	ADC_{P}[4] (%)	ADC_{GE}[5] (%)
Dissection	68.62 ± 2.61^{d}	86.77 ± 1.27^{b}	54.21 ± 3.37^{a}	70.45 ± 1.14^{d}
Sedimentation				
60 min	77.64 ± 2.06^{a}	89.84 ± 1.36^{ab}	19.59 ± 2.83^{d}	84.62 ± 0.93^{a}
120 min	75.81 ± 0.99^{ab}	91.10 ± 1.33^{a}	39.56 ± 2.51^{b}	82.72 ± 0.54^{ab}
240 min	72.82 ± 2.13^{bc}	87.71 ± 1.93^{ab}	30.38 ± 3.01^{c}	78.57 ± 1.91^{c}
480 min	71.11 ± 2.41^{dc}	90.74 ± 2.51^{a}	59.34 ± 3.25^{a}	81.15 ± 2.60^{b}
Regression[1]				
Linear	–	ns	P < 0.0001	ns
Quadratic	P < 0.0001	ns	ns	P < 0.0001
Broken-line	P < 0.0001	ns	ns	ns

Values in same column with different superscript letter are statistically different by SNK multiple range test at P < 0.05; ns – not significant.
[1]Regression analysis performed only on data of faeces collected at different times by sedimentation in the water column.
[2]Apparent digestibility coefficient of dry matter.
[3]Apparent digestibility coefficient of protein.
[4]Apparent digestibility of phosphorus.
[5]Apparent digestibility coefficients of gross energy.

Table 4
Apparent digestibility coefficients of nutrients and dry matter calculated from faeces collected by dissection in different gut sections of *Colossoma macropomum* fed diets containing graded levels of dietary binder (n = 3, mean ± SD)

		Apparent digestibility coefficient (%)		
Binder level (g kg^{-1})	Gut section	Dry matter	Phosphorus	Protein
0.0	Proximal	39.98 ± 2.82	49.92 ± 1.55 ^{cd}	70.69 ± 0.86^{bcd}
	Medium	39.13 ± 2.17	48.59 ± 6.02^{d}	70.26 ± 0.91 ^{cd}
	Distal	50.09 ± 5.07	53.11 ± 0.91 ^{cd}	77.45 ± 2.49^{b}
2.0	Proximal	40.10 ± 4.82	60.14 ± 0.95^{ab}	68.17 ± 1.71^{d}
	Medium	39.86 ± 7.57	54.76 ± 2.40^{bc}	71.93 ± 1.61^{bcd}
	Distal	50.84 ± 5.07	65.96 ± 3.10^{bcd}	75.10 ± 1.94^{bc}
4.0	Proximal	50.13 ± 2.81	52.34 ± 2.98 ^{cd}	73.95 ± 1.88^{bcd}
	Medium	50.89 ± 6.00	58.39 ± 3.90^{bcd}	73.46 ± 2.41^{bcd}
	Distal	74.06 ± 4.47	76.43 ± 1.65^{a}	85.76 ± 1.69^{a}
Effects (P value)				
Binder		<0.0001	<0.0001	<0.0001
Gut section		<0.0001	<0.0001	<0.0001
Binder × Gut		0.6384	0.0006	0.0176

Values in same column with different superscript letters are statistically different by Scheffé multiple range test at P < 0.05.

Among the three methods of faeces collection we attempted to evaluate, only two could be performed with *C. macropomum*: the dissection and the sedimentation in the water column. An insufficient amount of faeces was the result of using stripping, which may be related to the coiled end section of *C. macropomum* intestine, the rigid musculature in the abdominal cavity and the sensitivity of this species to handling. Although we could not find any report describing the digestive tract anatomy of *C. macropomum*, one study described the basic anatomy of the hybrid 'tambatinga' (*Colossoma macropomum* x *Piaractus brachypomus*), which seems to be very similar to *C. macropomum* (Ferreira et al., 2013).

As we expected, the ADC of nutrients from dissection were generally lower than the sedimentation method. Additionally, a high rate of nutrient leaching was observed when faeces were collected by the sedimentation method, probably due to the physical characteristics of *C. macropomum* faeces (faeces are generally less cohesive and dense, easily disintegrate with water movement and are similar to salmonid faeces). Phosphorus was the nutrient more prone to leaching, while protein seemed to not leach until 8 h in the water column.

ADC of P was similar between the sedimentation method at 480 min and the dissection method. This result cannot be explained since the duration of faeces in the water column led to an increase of P leaching and, thus, produced higher ADCs than the dissection method. The reduced volume of samples collected after the first hour and the possible inadequacy of chromium oxide as an inert marker for digestibility studies with *C. macropomum* may have affected the ADC of P in both methods, as reported previously for other species (Austreng et al., 2000). However, further studies are needed to evaluate the effect of different digestibility markers and

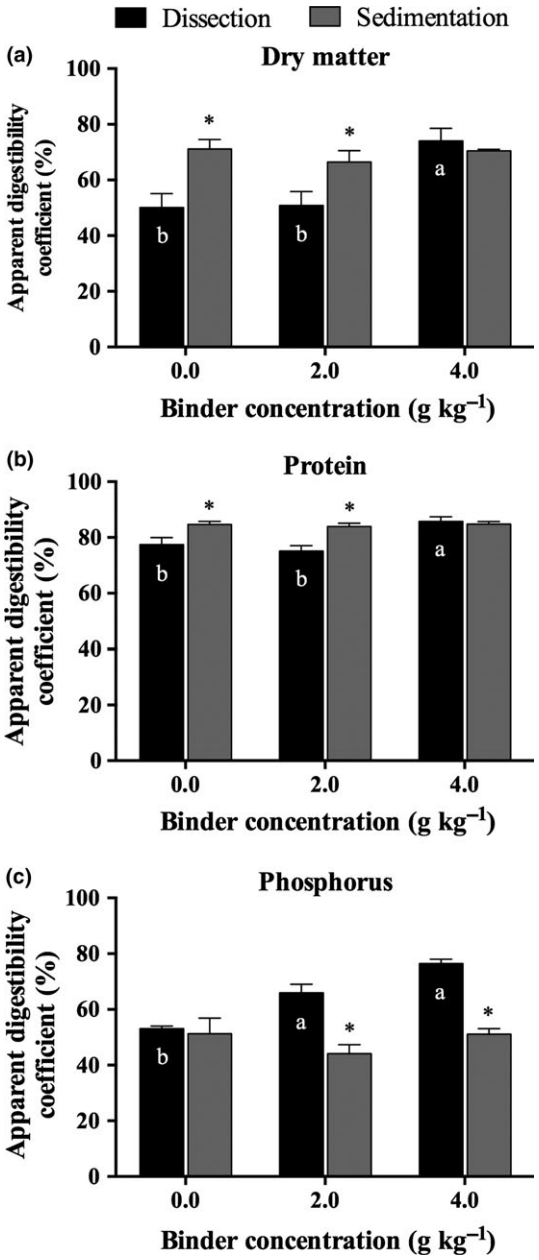

Fig. 2. Apparent digestibility of nutrients and dry matter calculated from faeces collected by sedimentation and dissection (hindgut), *Colossoma macropomum* juveniles fed diets containing graded levels of dietary binder. Asterisk = differences between methods in each dietary level of binder by F test at $P < 0.05$; letters inside bars = differences among dietary binder levels in each faeces collecting method by Scheffé multiple range test ($n = 3$, mean ± SD). Bar representing the sedimentation method = average (±SD) of three groups of fish with faeces pooled from six consecutive samplings; bar representing dissection = average (±SD) of three groups of fish with faeces from each fish pooled by group

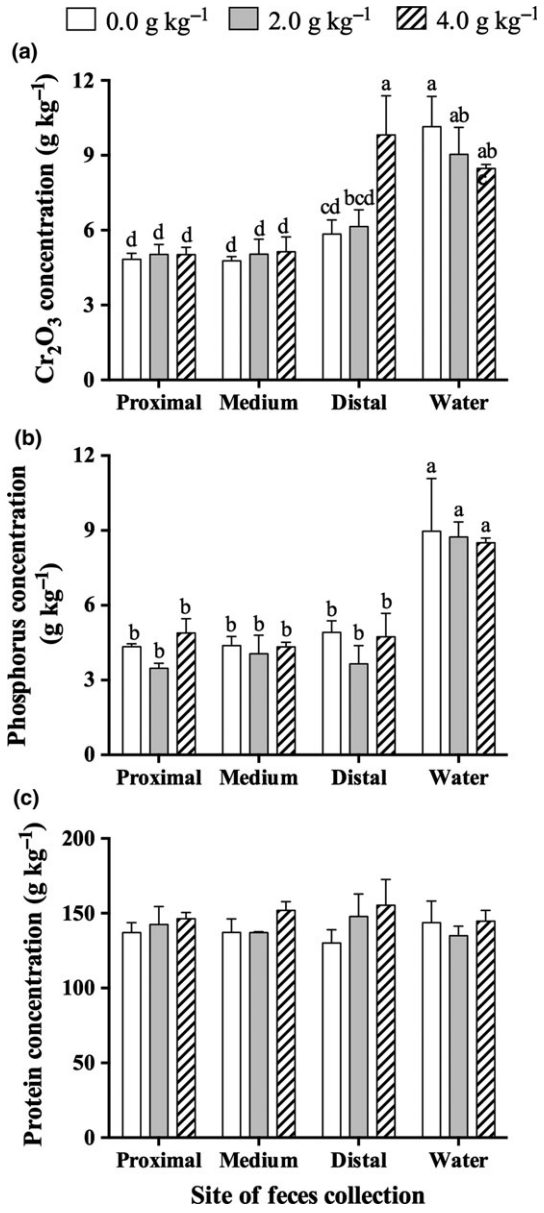

Fig. 3. Chromic oxide (a), phosphorus (b) and protein (c) concentration, *Colossoma macropomum* faeces collected in water column and at different gut sections of fish fed diets with graded levels of binder. Significant differences denoted by different letters at top of bars by Scheffé multiple range test. Bars of intestinal sections = average (±SD) of three groups of fish with faeces from each fish pooled by group; bars for water column = average (±SD) of three groups of fish with faeces pooled from six consecutive samplings

their concentration on ADC of nutrients for *C. macropomum*. Additionally, because we indirectly measured the P leaching using the ADC, this most likely affected the results since it takes a combination of errors during the sampling which are inherent to faeces collection.

Phosphorus is absorbed by passive and active transport across the luminal wall of fish enterocytes using a coupled Na-dependent transporter or by diffusion (Lall, 2002; Sugiura and Ferraris, 2004). However, the main site of absorption is species-specific and can occur along the intestinal tract or in specific gut sections, such as the pyloric caeca in salmonids (Sugiura and Ferraris, 2004; Hua and Bureau, 2010). To the best of our knowledge, there are no studies on mineral requirement and/or the mechanisms regulating P

absorption in *C. macropomum* or other related species. Thus, based on our results of P concentration in different segments of the digestive tract of *C. macropomum*, we may hypothesize that P absorption may occur throughout the intestinal tract since there were no differences in P concentration among the intestine sections (Fig. 3b). However, further studies must be performed to precisely indicate the mechanisms and site of P absorption in *C. macropomum*.

Our results clearly demonstrate that *C. macropomum* faeces are prone to nutrient and/or dry matter leaching in the water column, except for protein. Despite this constraint of the sedimentation method, the large number of animals needed to obtain significant amounts of samples to perform analysis in the dissection method reduces its applicability in animal studies, mainly considering the ethical issues. Additionally, the capacity of *C. macropomum* to digest and/or absorb nutrients until the last section of the gut (Table 4) increases the drawbacks of the dissection method.

Chime is a complex matter made up of diverse constituents, such as innate hydrocolloids and solutes. Interaction between the binder and these compounds may modify viscosity or gelation properties of polysaccharide binders, and thus the bolus (Brinker et al., 2005). Additionally, it is nearly impossible to model the interaction of the binder with the chime in the course of the alimentary tract due to the dynamic processes of digestion, absorption, and pH change (Brinker et al., 2005). Therefore, empirical studies are needed to evaluate the effect of binder on faecal properties based on its action on the digestive tract of the fish.

Dietary binder has been primarily used in feeds for crustaceans and molluscs using wet or moist fish feeds as a mean to reduce the leaching of nutrients from the feeds into the environment (Partridge and Southgate, 1999; Dominy et al., 2004; Genodepa et al., 2007; Rosas et al., 2008; Meyers and Zein-Eldin, 2009; Simon, 2009). Furthermore, current knowledge on dietary binders originated from these and previous investigations when pelleted feeds were the main type of diet used in fish production (Brinker et al., 2005). However, the use of dietary binders have been recently reemphasized as a mean to control the discharge of particulate faeces in salmonid production, showing prospective results to increase trout faecal stability (Brinker et al., 2005; Brinker, 2007, 2009; Unger and Brinker, 2013). Thus, we aimed to use this nutritional approach to reduce the leaching of nutrients in *C. macropomum* faeces and thus improve the sedimentation method application to collect faeces in digestibility trials for this species. Our results demonstrated that the commercial dietary binder used in this study (Nutri-Binder®) had limited capacity to reduce the leaching of P but reduced the leaching of dry matter and protein at the dietary level of 4.0 g kg^{-1}. Additionally, a linear reduction on ADC values and faeces nutrient concentration variability expands the application of dietary binders in fish nutrition studies, with emphasis on *C. macropomum*.

Different from results with salmonids (Brinker et al., 2005; Brinker, 2007), the effective level of commercial binder tended to increase the digestibility of nutrients (Fig. 2) and the transit time of the bolus (data not shown) for *C. macropomum*. Generally, the negative effect of binder is observed when high levels are used, usually hampering digestibility by increasing the bolus flow rate and reducing the time of chime particles are exposed to digestive enzymes (Amidon, 1985). However, we observed the opposite result in *C. macropomum* and based on previous works using binders (Cantelmo et al., 1999; Brinker et al., 2005), it is unlikely that the concentrations used in this study were high for fish. Two explanations for the high ADC found using the dissection method, irrespective of the intestinal section, may apply to our study: first, *C. macropomum* may be able to partially digest the binder used in this study; second, the gut microbiota may have used the binder as a prebiotic and increased health status of the digestive tract. Thus, further studies are necessary to determine whether these hypotheses may be applied to *C. macropomum*.

In summary, we demonstrated that *C. macropomum* faeces are prone to nutrient leaching when the sedimentation method of faeces collection is used; the dissection method may not be applicable to *C. macropomum* due to digestive tract morphology and the large number of animals required for the study, which diverge from the ethics in animal studies. As a means to reduce the variability and leaching of nutrients in *C. macropomum* faeces collected by sedimentation, we recommend the use of a dietary binder at 4.0 g kg^{-1} and/or the hourly collection of faeces. However, further studies are needed using other dietary binders, such as alginate and gum guar, as well as the evaluation of the physical properties of *C. macropomum* faeces to quantitatively determine the effect of binder on faeces stability and the digestive physiology of *C. macropomum*.

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References

Allan, G. L.; Rowland, S. J.; Parkinson, S.; Stone, D. A.; Jantrarotai, W., 1999: Nutrient digestibility for juvenile silver perch *Bidyanus bidyanus*: development of methods. Aquaculture **170**, 131–145.

Alliot, E.; Pastoreaudt, A.; Pelaez-Hudlet, J.; Métailler, R., 1979: Utilisation des farines vègetales et des levures cultivées sur les alcanes pour l'alimentation du bar (*Dicentrarchus labrax* L.). In: Proceedings of world symposium finfish nutrition and fishfeed technology, **vol. 2**, Heenemann, Berlin, pp. 229–238.

Amidon, G. L., 1985: Fluid mechanics and intestinal transit. Gastroenterol. **88**, 858.

Association of Official Analytical Chemists (AOAC), 1990: Official Methods of Analysis of the Association of Official Analytical Chemists, 16th edn (K. Helrich, Ed.), AOAC, Arlington, VA, pp. 1298.

Austreng, E.; Storebakken, T.; Thomassen, M. S.; Refstie, S.; Thomassen, Y., 2000: Evaluation of selected trivalent metal oxides

as inert markers used to estimate apparent digestibility in salmonids. Aquaculture **188**, 65–78.

Blyth, D.; Tabrett, S.; Bourne, N.; Glencross, B., 2015: Comparison of faecal collection methods and diet acclimation times for the measurement of digestibility coefficients in barramundi (*Lates calcarifer*). Aquac. Nutr. **21**, 248–255.

Bremer Neto, H.; Graner, C. A. F.; Pezzato, L. E.; Padovani, C. R., 2005: Determinação de rotina do crômio em fezes, como marcador biológico, pelo método espectrofotométrico ajustado da 1,5-difenilcarbazida (Chrome routine determination in faeces, as a biological marker, by the spectrophotometric method of 1,5-diphenylcarbazide). Cienc. Rural. **35**, 691–697.

Brinker, A., 2007: Guar gum in rainbow trout (*Oncorhynchus mykiss*) feed: the influence of quality and dose on stabilisation of faecal solids. Aquaculture **267**, 315–327.

Brinker, A., 2009: Improving the mechanical characteristics of faecal waste in *rainbow trout*: the influence of fish size and treatment with a non-starch polysaccharide (guar gum). Aquac. Nutr. **15**, 229–240.

Brinker, A.; Koppe, W.; Rösch, R., 2005: Optimised effluent treatment by stabilised trout faeces. Aquaculture **249**, 125–144.

Brown, P. B. 1993: Comparison of fecal collection methods for determining phosphorus absorption in rainbow trout. In: Fish nutrition in practice, IV international symposium on fish nutrition and feeding (S. J. Kaushik, P. Luquet, Eds), pp. 443–448. (Biarritz) France, June 24-27, 1991. Institut National de la Recherche Agronomique, Paris(Les Colloques no. 61).

Cantelmo, O. A.; Pezzato, L. E.; Barros, M. M.; Ribeiro, M. A. R., 1999: Influencia de diferentes aglutinantes na digestibilidade da matéria seca e da proteína bruta, no pacu (*Piaractus mesopotamicus*) arraçoado com rações elaboradas com e sem vapor (Influence of different binders in dry matter and crude protein digestibility in Pacu (*Piaractus mesopotamicus*) fed diets elaborated with or without steam). Acta Scient. **21**, 277–282.

Cho, C. Y.; Cowey, C. B.; Watanabe, T., 1985: Methodological approaches to research and development. In: Finfish nutrition in Asia. C. Y. Cho, C. B. Cowey and T. Watanabe (Eds) Ottawa, Canada, IDRC, pp. 10–80.

Choubert, G.; De la Noüe, J.; Luquet, P., 1982: Digestibility in fish: improved device for the automatic collection of feces. Aquaculture **29**, 185–189.

De La Noüe, J.; Choubert, G., 1986: Digestibility in rainbow trout: comparison of the direct and indirect methods of measurement. Prog. Fish. Cult. **48**, 190–195.

Dominy, W. G.; Cody, J. J.; Terpstra, J. H.; Obaldo, L. G.; Chai, M. K.; Takamori, T. I., 2004: A comparative study of the physical and biological properties of commercially-available binders for shrimp feeds. J. Appl. Aquac. **14**, 81–99.

Ferraris, R. P.; Catacutan, M. R.; Mabelin, R. L.; Jazul, A. P., 1986: Digestibility in milkfish, *Chanos chanos* (Forsskal): effects of protein source, fish size and salinity. Aquaculture **59**, 93–105.

Ferreira, A. H. C.; Alencar-Araújo, M. N. B.; Guerra, S. P. L.; Lopes, J. B.; Araripe, H. G. A.; Andrade, F. T.; Santana, H. A. Jr, 2013: Anatomia do aparelho digestório do tambatinga (Digestive tract anatomy of tambatinga). Revista Eletrônica Nutritime **10**, 2501–2512 (Available at: http://www.nutritime.com.br/arquivos_internos/artigos/artigo_203.pdf).

Genodepa, J.; Zeng, C.; Southgate, P. C., 2007: Influence of binder type on leaching rate and ingestion of microbound diets by mud crab, *Scylla serrata* (Forsskål), larvae. Aquac. Res. **38**, 1486–1494.

Glencross, B. D.; Booth, M.; Allan, G. L., 2007: A feed is only as good as its ingredients – A review of ingredient evaluation for aquaculture feeds. Aquac. Nutr. **13**, 17–34.

Guimarães, I. G.; Pezzato, L. E.; Barros, M. M., 2008a: Amino acid availability and protein digestibility of several protein sources for Nile tilapia, *Oreochromis niloticus*. Aquac. Nutr. **14**, 396–404.

Guimarães, I. G.; Pezzato, L. E.; Barros, M. M.; Tachibana, L., 2008b: Nutrient digestibility of cereal grain products and by-products in extruded diets for nile tilapia. J. World. Aquac. Soc. **39**, 781–789.

Halver, J. E., 1989: Fish nutrition, 2. Academic Press, San Diego, CA. 798 pp.

Henken, A. M.; Kleingeld, D. W.; Tijssen, P. A. T., 1985: The effect of feeding level on apparent digestibility of dietary dry mater, crude protein and gross energy in the African catfish (*Clarias gariepinus*). Aquaculture **51**, 1–11.

Hua, K.; Bureau, D. P., 2010: Quantification of differences in digestibility of phosphorus among cyprinids, cichlids, and salmonids through a mathematical modelling approach. Aquaculture **308**, 152–158.

Lall, S. P., 2002: The minerals. In: Fish nutrition (J. E. Halver, R. W. Hardy, Eds), 3rd edn. pp. 260–301, Academic Press, New York.

Meyers, S. P.; Zein-Eldin, Z. P., 2009: Binders and Pellet Stability in Development of Crustacean Diets. Proc. Annu. Work. - World. Maric. Soc. **3**, 351–364.

NRC 2011: Nutrient requirements of fish and shrimp. National Academic Press, Washington.

Oishi, C. A.; Nwanna, L. C.; Pereira Filho, M., 2010: Optimum dietary protein requirement for Amazonian Tambaqui, *Colossoma macropomum* Cuvier, 1818, fed fish meal free diets. Acta Amazon **40**, 757–762.

Partridge, G. J.; Southgate, P. C., 1999: The effect of binder composition on ingestion and assimilation of microbound diets (MBD) by barramundi *Lates calcarifer* Bloch larvae. Aquac. Res. **30**, 879–886.

Portz, L., 2001: Recentes avanços na determinação das exigências e digestibilidade da proteína e aminoácidos em peixes (Recent advances in determining the requirements and digestibility of protein and amino acids in fish). In: Anais da Reunião Anual da Sociedade Brasileira de Zootecnia, Piracicaba (Proceedings of the Annual Meeting of the Brazilian Society of Animal Science, Piracicaba). Piracicaba, Brazil. pp. 528–542.

Rosas, C.; Tut, J.; Baeza, J.; Sanchez, A.; Sosa, V.; Pascual, C.; Arena, L.; Domingues, P.; Cuzon, G., 2008: Effect of type of binder on growth, digestibility, and energetic balance of *Octopus maya*. Aquaculture **275**, 291–297.

Santos, J. G. A., 2012: Exigência em fósforo digestível para o tambaqui (*Colossoma macropomum*) (Digestible phosphorus requirement for tambaqui, *Colossoma macropomum*). PhD Thesis, Universidade Federal de Goiás, Brazil, 109 pp.

Shearer, K. D., 2000: Experimental design, statistical analysis and modelling of dietary nutrient requirement studies for fish: a critical review. Aquac. Nutr. **6**, 91–102.

Silva, J. D.; Pereira-Filho, M.; Oliveira-Pereira, M. I. D., 1999: Digestibility of seeds consumed by tambaqui (*Colossoma macropomum* Cuvier, 1818): an experimental approach. Biology of tropical fishes (A. L. Val, V. M. Almeida-Val, Eds). INPA, Manaus, pp. 137–148.

Simon, C. J., 2009: The effect of carbohydrate source, inclusion level of gelatinised starch, feed binder and fishmeal particle size on the apparent digestibility of formulated diets for spiny lobster juveniles, *Jasus edwardsii*. Aquaculture **296**, 329–336.

Sugiura, S. H.; Ferraris, R. P., 2004: Contributions of different NaPi cotransporter isoforms to dietary regulation of Ptransport in the pyloric caeca and intestine of rainbow trout. J. Exp. Biol. **207**, 2055–2064.

Unger, J.; Brinker, A., 2013: Floating feces: a new approach for efficient removal of solids in aquacultural management. Aquaculture **404–405**, 85–94.

Vens-Cappell, B., 1985: Methodical studies on digestion in trout. 1. Realiability of digestion coefficients in relation to methods for faces collection. Aquac. Eng. **4**, 33–49.

Windell, J. T.; Foltz, J. W.; Sarokon, J. A., 1978: Methods of fecal collection and nutrient leaching in digestibility studies. Prog. Fish Cult. **40**, 51–55.

Author's address: Igo Guimarães, Laboratório de Pesquisa em Aquicultura – LAPAQ, Universidade Federal de Goiás – Regional Jataí, Rodovia BR 364, Km 195, Campus Cidade Universitária, 75801-615 Jataí, GO, Brazil.
E-mail: \igoguimaraes@ufg.br