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Dietary protein and body mass affect ammonium excretion in white cachama *(Piaractus brachypomus)*¤

La proteína dietaria y la masa corporal afectan la excreción de amonio en cachama blanca (Piaractus brachypomus)

A proteína dietética e massa corporal afeta a excreção de amônio em pirapitinga (Piaractus brachypomus)

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Summary

Background: ammonia (NH_3) is the main excretion product from protein catabolism in fish, eliminated primarily through the gills. The proportion excreted by each species depends on factors such as protein quality, energy level and diet balance, body size of the animals, and environmental factors such as water temperature and pH. **Objective:** to determine the effect of dietary protein level (D1 = 250 g/kg, D2 = 300 g/kg, D3 = 350 g/kg) and body weight (P1 = 45 g, P2 = 250 g, P3 = 520 g) on ammonia excretion (AE) in white cachama (*Piaractus brachypomus*). **Methods:** basal AE level was determined by measuring water ammonia concentration every 2 h for 26 h after a 48 h fasting period. The AE in response to CP levels was determined for each fish size by measuring ammonia every 2 h for 26 h, after feeding them to satiety with the experimental diets. **Results:** basal AE was 177.2, 128.7, and 79.2 mg $N\text{-}NH4^+$/day/kg live weight (LW) for P1, P2, and P3, respectively. The differences between treatments were significant ($p<0.05$). The AE rate, depending on protein level and body weight, was significantly different for all comparisons ($p<0.05$), similar to the comparison of main effects. **Conclusion:** the lightest fish and the highest protein content intake increased ammonium excretion.

Key words: *basal excretion, characids, crude protein, postprandial pulse.*

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Resumen

Antecedentes: el amonio (NH_3) es el principal producto de excreción resultante del catabolismo proteico en peces. Su proporción está determinada por la calidad del alimento, el balance proteína/energía de la dieta, el tamaño del pez, y por factores ambientales como temperatura y pH del agua. **Objetivo:** determinar el efecto del nivel de proteína de la dieta (D1 = 250, D2 = 300 y D3 = 350 g PC/kg) y del peso corporal (P1 = 45, P2 = 250 y P3 = 520 g de peso individual) sobre las tasas de excreción de amonio (TEA) en juveniles de cachama blanca (*Piaractus brachypomus*). **Metodología:** la TEA basal fue determinada midiendo la concentración de amonio en el agua cada 2 h durante 26 h posteriores a un periodo de ayuno de 48 h. La TEA en función de los niveles de PC y para cada peso corporal fue determinada midiendo el amonio cada 2 h durante 26 h, después de alimentar a saciedad con las dietas experimentales. **Resultados**: la TEA basal mostró valores de 177,2 para P1, 128,7 para P2 y 79,2 para P3 expresados en mg $N\text{-}NH_4^+$/día/kg de peso vivo (PV); las diferencias entre tratamientos fueron estadísticamente significativas ($p<0,05$). El análisis de las tasas de excreción en función del nivel de proteína y del peso corporal, mostró diferencias significativas entre todas las comparaciones ($p<0,05$); igual ocurrió en la comparación de los efectos simples. **Conclusión:** a menor peso individual y a mayor tenor proteico, mayor excreción de amonio en cachama blanca.

Palabras clave: *carácidos, excreción basal, proteína cruda, pulso postprandial.*

Resumo

Antecedentes: o amônio (NH_3) é o principal produto de excreção que resulta do catabolismo proteico dos peixes. A proporção do amônio é determinada pela qualidade do alimento fornecido, do balanço entre proteína e energia na dieta, do tamanho corporal do peixe e de fatores ambientais como a temperatura e o pH da água. **Objetivo:** determinar o efeito do nível da proteína na dieta (D1 = 250, D2 = 300 e D3 = 350 g PC/kg) e do peso corporal (P1 = 45, P2 = 250 y P3 = 520 g de peso individual) na taxa de excreção de amônio (EA) em juvenis de pirapitinga (*Piaractus brachypomus*). **Métodos**: a taxa basal de excreção de amônio foi determinada medindo a concentração de amônio na água a cada 2 h até as 26 h. Está medição se fez depois de deixar os peixes num de jejum de 48 h. A excreção do amônio se fez em função dos níveis de PC e para cada peso corporal foi determinada medindo o amônio a cada 2 h durante 26 h depois de alimentar a saciedade com as dietas experimentais. **Resultados:** a excreção de amônio basal mostrou valores de 177,2 para P1, 128,7 para P2 e 79,2 para P3 expressados em mg $N\text{-}NH_4^+$/dia/kg de peso vivo (PV); as diferenças entre tratamentos foram estatisticamente significativas ($p<0,05$). As análises das taxas de excreção em função do nível de proteína e do peso corporal mostraram diferenças significativas entre todas as comparações ($p<0,05$); igual resultado foi observado quando comparadas as diferenças entre os efeitos simples. **Conclusão:** ao ter menor peso corporal e maior teor de proteína na dieta, aumenta a taxa de excreção basal de amônio em juvenis de pirapitinga.

Palavras chave: *caracídeos, excreção basal, proteína bruta, pulso pós-prandial.*

Introduction

Data from FAO (2010) show that the accelerated growth of aquaculture in recent years has increased the use of industrialized feed, and thus the waste excreted to the environment (Gelineau *et al*., 1998; Sagratzki *et al*., 2004). Such waste materials can amount up to 75% of the consumed feed (Jimenez-Montealegre *et al*., 2005; Kobayashi *et al*., 2007). Ammonia (NH_3), the main excretion product of protein catabolism, is eliminated primarily through the gills. The proportion excreted by each species depends on factors such as protein quality, energy level, and diet balance. It also depends on the body size of the animals and environmental factors such as water temperature (Fu-Guang *et al*., 2009; Kieffer and Wakefield, 2009) and pH (Green and Hardy, 2008; Peres and Oliva-Teles, 2006).

Unlike terrestrial vertebrates, fish tend to oxidize a great part of dietary amino acids to obtain energy (Gao *et al*., 2005; Guillaume *et al*., 2004; Lim *et al*., 2001; Nyina-Wamwiza *et al*., 2005). A direct relationship between dietary protein and ammonium excretion has been observed (Buttle *et al*., 1995; Chakraborty and Chakraborty, 1998; Engin and Carter, 2001). According to Jobling (1981b), Dosdat *et al*. (1996), García-Gallego *et al*.(1999), Peres and Oliva-Teles (2006), as excretion increase starts briefly after feed is offered (postprandial pulse) and continues for one to four days. It then declines smoothly to its original or baseline levels. Even though excretion pattern is a function of the quantity and balance of essential amino acids (Saavedra *et al.*, 2009) and the protein/energy proportion in the diet, it is also related to fish species (Oliva-Teles *et al*., 2006) and feeding schedule (Gelineau *et al.*, 1998; Wicks *et al*., 2002; Zakes *et al*., 2006), among other factors.

White cachama, *Piaractus brachypomus* (Cuvier, 1818), is widely distributed in the Amazon and Orinoco regions. This species has excellent zootechnical features. It can be produced in captivity using concentrate feeds (Vásquez-Torres, 2005). It is currently the most abundant native fish produced in Colombia, with 4,399 TM in 2010 (CCI, 2010). The present study was designed to determine ammonia excretion rates in response to dietary protein level for three weights of white cachama, *Piaratus brachypomus*.

Materials and methods

Ethical considerations

This research was approved by the Ethics Committee for Animal Research of Universidad de los Llanos, Villavicencio (003 Act of November 18, 2009).

Location

The study was conducted at the Instituto de Acuicultura (IALL) of Universidad de los Llanos (IALL) in Villavicencio (Meta, Colombia), located at 4°04'24"N, 73°34'56"W.

Biological material

Three groups of young white cachamas were used, disregarding sexual differentiation. The fish were obtained through inducted reproduction at the IALL Fishing Station. The animals were transferred from the cultivation ponds to circular cement tanks (3000 L) with constant water flow (5 L/s) and permanent airing to acclimate them to the experimental conditions. Average weight in each group was P1 = 46.07 ± 4.36 g, P2 = 253.33 ± 3.35 g, and P3 = 520 ± 5.0 g. The amount of water was kept within the comfort range for this species throughout the experimental period as described by Vásquez-Torres (2005). During the adaptation to the laboratory conditions, fish were fed once a day (at 9:00 am) to apparent satiety following the semi-purified reference diet (SRD) developed by Vásquez-Torres *et al.* (2002) (Table 1).

Experimental diets

Three experimental diets were formulated based on SRD. Crude protein (CP) level in the diets was 250 g/kg (D1), 300 g/kg (D2), and 350 g/kg (D3) (Table 1). These levels were achieved by modifying the concentration of the protein sources (casein and gelatin) while maintaining the proportions between them to guarantee the essential amino acid balance according to the method proposed by Vásquez-Torres (2001). The levels of the other energy sources (dextrin and oils) were also adjusted to keep diets isocaloric (approximately 13.5 kJ/kg digestible energy, DE), as calculated from physiological values proposed for fish in general (De silva and Anderson, 1995). Concentration of the other ingredients was kept constant across diets, and the final adjustments were achieved by varying cellulose and carboxymethyl-cellulose (CMC) inclusion. Proximal composition was verified following AOAC (1995).

Physical-chemical conditions of the experimental units

The experimental work was conducted in the nutrition bio testing laboratory of IALL using nine 40 L effective capacity plastic recipients filled with mechanically filtered water from a recycling system and four sequenced bio-filters to keep water under stable conditions within comfort ranges for the species: pH 6.75 ± 0.19, 26.1 ± 0.47 ºC, 6.6 ± 0.34 mg/L dissolved oxygen (DO), and ammonium in values under 0.001 mg L^{-1}. Each tank had a device for permanent airing and a hydraulic device to ease the water change in approximately 100% during a very short period (5 minutes) with no stress for the fish. The DO, T ºC, and pH were controlled with a Thermo Orion probe (Waltham, MA, USA) and ammonium measurements were taken with an Ammonium Spectroquant® Ref. 1.14752.000 kit (phenol-hypochlorite method) (Merck KGaA, Darmstadt, Germany) and a spectrophotometer with a sensitivity of 0.001 mg/L with a rank of 0.001 to 5 mg/L N-NH3+NH4+ (Merck, Merk Spectroquant NOVA 60, Darmstadt, Germany).

Table1. Ingredients (g/kg DM) and proximal analysis of Semipurified Reference Diet (SRD) and experimental diets fed to white cachama (*Piaractus brachypomus*).

Ingredients	SRD	Experimental diets		
		D1	D2	D3
Casein[1]	300.0	280.0	336.0	392.0
Gelatin[2]	50.0	33.8	40.6	47.3
Dextrin	300.0	400.0	340.0	280.0
Fishoil	30.0	20.0	17.0	14.0
Cornoil	30.0	20.0	17.0	14.0
Vitamin premixture[3]	1.0	1.0	1.0	1.0
Micromineral mixture[4]	0.5	0.5	0.5	0.5
Mineral mixture[5]	40.0	40.0	40.0	40.0
VitaminC (StayC-35)	0.3	0.3	0.3	0.3
Carboxymethyl-Cellulose (CMC)	68.2	32.0	32.0	32.0
Cellulose	180.0	172.4	175.6	178.9
Calculated digestible energy (kJ/kg)[6]	12.4	12.3	12.8	12.7
Proximate composition (*g*/kg*DM*)[7]				
Crudeprotein (Nx6.25)	336.1	256.1	304.7	350.3
Ether Extract	35.0	32.0	28.0	27.0
Ash	36.0	37.0	38.0	39.0
Dry matter	842.0	815.0	861.0	905.0

[1] Casein composition: MS 930; CP 864.2, Lipids 22.9, Ash 36.6 (g/kg DM); AAE composition (g/kg CP): Met 29.1, Lys 80.6, Thr 41.4, Arg 34.3, I-leu 50.2, Leu 93.4, Val 65.4, His 31.0, Phe 50.2.

[2] Gelatin composition: MS 910; CP 940.2 (g/kg DM); AAE composition (g/kg CP): Met 09.2, Lys 38.1, Thr 18.7, Arg 82.5, I-leu 15.2, Leu 30.2, Val 23.7, His 07.2, Phe 20.7.

[3] Rovimix vitamin compositionÒ (Lab. Roche S.A): Vit. A $8x10^6$ UI; Vit. D_3 $1.8x10^6$ UI, Vit. E 66.66 g, Vit. B_1 6.66 g, Vit. $B_2$13.33 g, Vit. B_6 6.66 g, Pantothenic acid 33.33 g, Biotin 533.3 mg, Folic acid 2.66 g, Ascorbic acid 400.0 g, Nicotinic acid 100.0 g, Vit. B_{12} 20.0 mg, Vit. K_3 6.66 g, filer csp. 1.0 kg.

[4] Composition of 100 g mineral mixÒ (Lab. Roche S.A.): Mg 1.0, Zn 16.0, Fe 4.0, Cu 1.0, I 0.5, Se 0.05, Co 0.01.

[5] Composition of 100 g macro-minerals mixture: $Ca(H_2PO_4)$ 13. 6 g; Ca Lactate 34.85 g; $2MgSO_4.7 H_2O$ 13.2 g; KH_2PO_4 24 g; NaCl 4.5 g; $AlCl_3$ 0.015 g, CMC 9.835 g.

[6] Calculated from physiological values: 1.9 kJ/kg for protein, 3.6 kJ/kg for lipids, and 1.50 kJ/kg for carbohydrates (De silva and Anderson, 1995).

[7] Average value of three samples.

Experimental procedure

Determination of the Basal Excretion Rate (BER). After an adaptation period of 15-days to the laboratory conditions and SRD, animals were separated by weight into three groups: 45 g (P1), 250 g (P2), and 500 g (P3) and allocated to experimental tanks at 12.5 g/L density (approximately 520 g per tank) to have three treatments and three replications per treatment.

Water was sampled (5 ml per tank) for ammonium concentration after fasting fish for 48 hours. Then, water in each unit was substituted with ammonium-free water. To reduce the stress produced by this activity, each container maintained a minimum of 20 cm water column, with water flowing in and out at the same speed during 8 to 10 seconds, enough to guarantee total water substitution. A second sample was taken two hours later, repeating the water substitution procedure. This procedure was repeated every two hours during 26 hours in order to determine the BER. The data of each repetition were registered for each group of animals according to the weight treatment. At the end, the average of all samples was calculated per replication to determine the amount of $N\text{-}NH_4^+$ (mg/day/kg LW), and its equivalence was established according to the water volume (40 L), time (24 hours), and live weight (1 kg).

Quantification of ammonium excretion asN-NH_4. An AxB factorial arrangement of treatments was used to compare the effects of body weight (P1, P2, P3) and protein level (D1, D2, D3) on ammonium excretion rate (AER). In a first stage all fish (P1, P2, and P3) were fed D1 to satiety once a day (at 9:00 am) for three days maintaining the experimental conditions previously described. On the third day, rejected feed was retired from the units (10 minutes after feeding), water renewal was performed, and the first samples were taken from each container to determine initial ammonium level. Two hours later, new samples were taken to determine excretion values during that period, followed again by water renewal. This sampling and renewal procedure was repeated every two hours during 26 hours until 13 samples (in triplicate) were obtained per treatment. The same protocol was repeated for diets D2 and D3 once the experiment was completed for D1.

Statistical analysis

Assumptions of normality, independence, experimental errors randomness, variance homogeneity, and medians and variances independence were verified. Ammonium

BER weights of three treatments were compared through ANOVA ($p<0.05$ significance level). The AER results were also compared through one-way variance analysis ($p<0.05$) for main effects and through the general linear model (GLM) for body weight and protein interactions for nine treatments. The model was $Y = \mu + P_i + CP_j + (PxCP)_{ij} + e_{ij}$, where Y_{ij} is the observed value, μ is the general median of the characteristic, P_i is the effect of weight for $_i$ = 46.1, 253.3 and 520.0 g, CP_j is the crude protein effect, being $_j$ = 256, 304 and 350 g/kg, $(PxCP)_{ij}$ is the deviation due to P and CP interaction, and e_{ij} is the error. Mean differences were determined with the Tukey test ($p<0.05$). Statistical analyses were performed with the SAS program (v8.2 Inc., USA).

Results

Excretion rates increased as protein levels increased, and decreased with increasing fish weight. According to table 2, the highest average value and the top excretion peaks were 42.49 mg N-NH_4^+/day/kg LW (significantly different from the value for 250 g of CP/kg at the baseline for the same weight) and 62.71 mgN-NH_4^+/day/kg LW, respectively, corresponding to P1 with 350 g/kg CP in the diet, while the lower ones were 27.04 (only ones that differed from the baseline) and 8.21 for P3 with 256 g/kg CP. Basal excretion was inversely correlated with body weight, with 13.63 mgN-NH_4^+/day/kg LW for P1 (highest value) and 6.09 mgN-NH_4^+/day/kg LW for P3 (lowest value), and all cases within treatment were significantly different. Diet differences were only found for P1, including basal excretion.

Basal ammonium excretion in mg N-NH_4^+/day/kg of LW

The basal excretion of ammonium among animals during 24 hours was statistically different. Excretion for P1 was 1.37 times higher than it was for P2, and 2.23 times higher in comparison to P3. The basal excretion rank (mg N-NH_4^+/day/kg LW) during the sampling period oscillated between 4.36 for P3 as the minimum value and 19.05 as the maximum for P1 (Table 2). Figures 1A, 1B, and 1C (dashed gray line) show a relatively stable behavior concerning the basal excretion during 24 hours.

Table 2. Ammonium excretion of juvenile white cachamas fed three protein levels during 24 hours (n = 39).

			Ammonium levels (mg N-NH_4^+/kg LW)		
Weight (g)	**CP g/kg**	**24 hours (mg N-NH4+/kg)**	**Minimum**	**Maximum**	**Average**
46	250	452.4 ± 5.5 c	11.64 ± 2	51.33 ± 3.21	34.8 ± 1.26 b
	300	512 ± 3.63 b	14.02 ± 4.37	52.92 ± 2	39.38 ± 2.48 a
	350	552.2 ± 9.3 a	24.08 ± 1.21	62.71 ± 1.37	42.49 ± 2.6 a
	Basal	117.8 ± 9.79 d	8.37 ± 1.37	19.05 ± 1.58	13.63 ± 1.36 c
253	250	427.9 ± 14.6 b	20.65 ± 6.19	39.19 ± 4.08	32.91 ± 2.26 b
	300	453.8 ± 8 b	20.66 ± 5.37	45.56 ± 4.07	34.91 ± 1.96 b
	350	505.4 ± 11.7 a	7.68 ± 1.21	51.65 ± 2.86	38.88 ± 2.06 a
	Basal	128.8 ± 6.74 c	6.76 ± 1.68	12.72 ± 3.37	9.9 ± 1.29 c
520	250	351.5 ± 6.3 b	8.21 ± 0.44	34.61 ± 2.77	27.04 ± 1.78 a
	300	374.3 ± 7.7 a	13.07 ± 2.03	37.43 ± 1.93	28.79 ± 1.02 a
	350	385.6 ± 10.4 a	15.64 ± 2.47	43.84 ± 1.53	29.66 ± 1.15 a
	Basal	79.2 ± 5.43 c	4.36 ± 0.44	8.97 ± 0.44	6.09 ± 0.94 b

Values represent the average of three repetitions per treatment ± ED. Means in the same column with different superscript letter are significantly different (Tukey test; $p<0.05$).

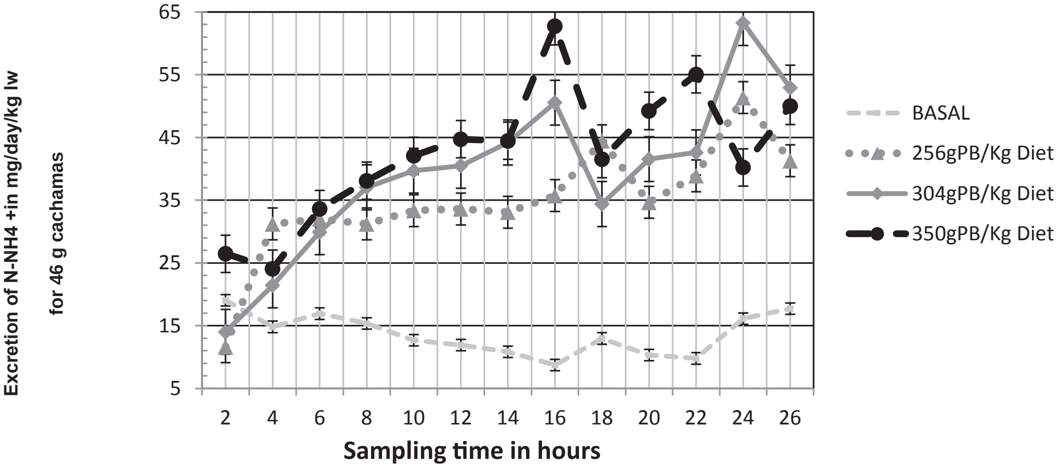

Figure 1A. Basal and post-feeding ammonium excretion of cachamas (*Piaractus brachypomus,* 46 g LW) fed 256, 304, and 350 g/kg protein.

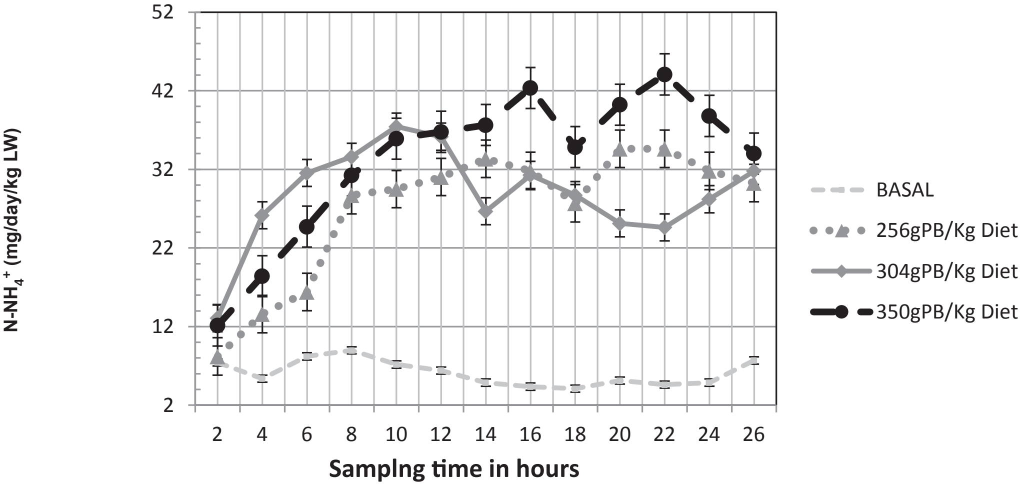

Figure 1B. Basal and post-feeding ammonium excretion of white cachamas (*Piaractus brachypomus;* 253 g LW) fed 256, 304, and 350 g/kg protein.

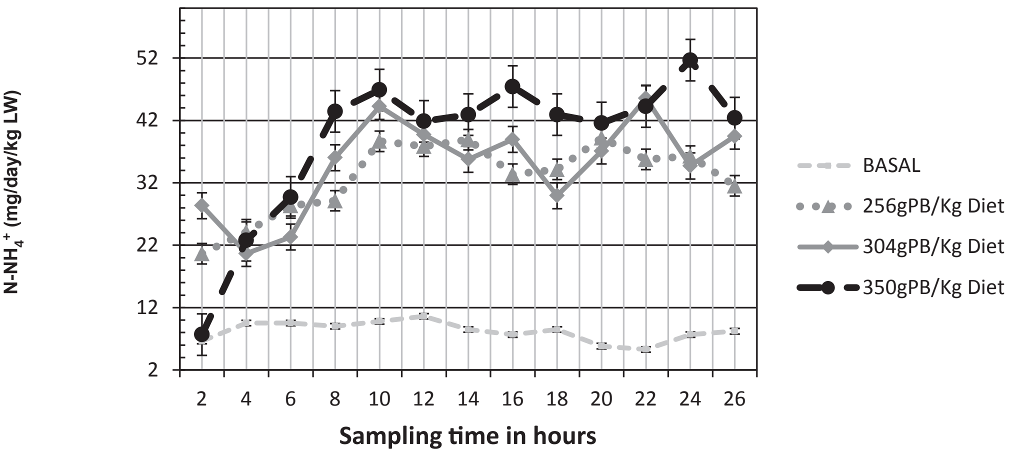

Figure 1C. Basal and post-feeding ammonium excretion of white cachamas (*Piaractus brachypomus;* 520 g LW) fed 256, 304, and 350 g/kg protein.

Ammonium excretion for P1 in connection with CP level

Postprandial (post-feeding) pulse was assessed during hour 24 for diets D1 and D2, and at hour 16 for diet D3 (Figure1A). The excretion increase in D3 relative to D1 was 1.2 times and 1.07 compared to D2. The minimum value found was 11.64 mg N-NH4+/day/kg LW for D1 and the maximum was 62.71 for D3 (Table 2). Total daily excretion was 452.4, 512.0 and 552.2 for D1, D2 and D3, respectively, with significant differences between treatments ($p<0.05$) (Table 3). Values did not return to the baseline after 26 hours of sampling in any of the cases.

Ammonium Excretion for P2 in connection with CP level

Similar to P1 with 350 g CP/kg (D3), the maximum excretion peak was reached at the 16th hour. The increase took place earlier than it did in P1 for D1 and D2. D1 reached its maximum value at the 10th hour and then remained relatively constant until the last sampling, while D2 had its top value at the 22nd hour (Figure 1B). For D3, 1.18 more ammonium was excreted than it was in D1, and 1.1 times more than it was in D2. The lowest and highest excretion values were for D3 (Table 2). Significant differences in daily excretion levels ($p<0.05$) occurred only between D3 and the other treatments (Table 3).

Ammonium Excretion for P3 in connection with CP level

Excretion rates increased more rapidly than they did in P1 and P2, except for D3. The highest excretion peak appeared in D3 at the 24th hour. For D2, the maximum pulse appeared at the 10th hour, and for D1 it appeared around the 20th hour (Figure 1C). The maximum value found in P3 (43.84 ± 1.53 mg N-NH$_4^+$/day/kg LW) was 1.4 times lower than that found in P1 (62.71 ± 1.37 mg N-NH$_4^+$/day/kg LW). Total daily excretion was significantly lower in D1 compared to D2 and D3 (Table 3).

There was always a statistical difference when each protein level was compared to the different weights (Table 3). The same happened with the main effects concerning weight and protein levels, keeping in mind that the excretion values directly increased with the increase of protein content, and diminished as weight augmented.

Table 3. Ammonia excretion (mg N-NH$_4^+$/day/kg LW), individual weight (P1, P2, P3), and dietary protein level (D1, D2, D3) in white cachamas (n = 3 for interactions and n = 9 for main effects)*.

	Weight x protein interaction			
	P1	**P2**	**P3**	**CP**
D1	452.4 ± 5.5^{Ca}	427.9 ± 14.6^{Bb}	351.5 ± 6.3^{Bc}	410.6 ± 52.6^{C}
D2	512.0 ± 3.6^{Ba}	453.8 ± 8.0^{Bb}	374.3 ± 7.7^{Ac}	446.7 ± 69.1^{B}
D3	552.2 ± 9.3^{Aa}	505.4 ± 11.7^{Ab}	385.6 ± 10.4^{Ac}	481.1 ± 85.9^{A}
Weight	505.6 ± 50.1^{a}	462.4 ± 39.4^{b}	370.5 ± 17.3^{c}	↑ ← Main effects

*The values represent the mean of three repetitions per treatment ± ED. Means in the same column with different superscript letter are significantly different (Tukey test; $p<0.05$). Lines: effect of weight in diet (lower case). Columns: effect of diet in weight (uppercase).

The variance analysis for the combined effects of Weight x Protein level was highly significant ($p<0.0001$; $R^2 = 0.98$). This result indicates a close interaction between these independent variables.

Discussion

Fish use predominantly protein as a metabolic subtract to obtain energy (Jobling, 1981a) through amino acid deamination. The final product of this metabolic pathway is ammonium (Chakraborty and Chakraborty, 1998), a compound that is excreted through the gills. Ammonium excretion can be used as an indicator of the effects of several nutritional and environmental factors on protein metabolism. It can also reflect general tendencies in the metabolic rate of fish (Jobling, 1981b). Furthermore, knowledge of ammonium excretion levels is vital to maintain optimal environmental conditions in fish farm sand to monitor the effluents discharged into the natural environment (Jatteau, 1997; Shuenn-Der *et al*., 2002). The excretion values obtained after the fasting period (at least 48 hours) are related to endogenous nitrogen excretion (Jobling, 1981b). Table 4 shows rates of basal excretion in several species, plus the data from this study.

After the feeding period, ammonium excretion rates of *Piaractus brachypomus* increased as protein level increased, and were up to four times higher in comparison to the baseline. As previously explained, the amount of dietary protein is the most determining factor for nitrogen excretion, particularly in the form of ammonium (Buttle *et al.*, 1995; Kelly and Kohler, 2003). Studies with fresh water fish fed diets with variable protein levels (from 250 to 550 g/kg CP) have reported ammonium excretion rates (mg NH3/day/kg LW) far above the values observed in the present study. Excretion values from 741.6 to 1096.8 were reported for Indian carps (*Labeo rohita*) fed diets with 230 to 380 g/Kg CP (Chakraborty and Chakraborty, 1998). Values between 572 and 1225 were reported for eels (*Anguilla australis australis*) fed between 250 and 550 g/kg CP (Engin and Carter, 2001), and between 1615.2 and 2018.4 for marine red drum fish (*Sciaenops ocellatus*) fed diets with 350 and 450 g/kgCP (Webb and Gatlin III, 2003). Similar results have been reported regarding ammonium excretion increase in connection with protein levels for fresh water species such as the common carp (*Cyprinus carpio*) (Chakraborty *et al.*, 1992), trout (*Oncorhynchus mykiss*) (Cheng *et al.*, 2003), and African catfish (*Clarias gariepinus*) (Buttle *et al.*, 1995) and also for sea water species such as the red drum (*Sciaenops ocellatus*) (McGoogan and Gatlin III, 1999), European sea bass (*Dicentrarchus labrax*) (Peres and Oliva-Teles, 2001), bass (*Bidyanus bidyanus*) (Yang *et al.*, 2002), porgy (*Sparus aurata*) (Martínez, 2002), and milkfish (*Chanos chanos*) (Sumagaysay-Chavoso, 2003).

Table 4. Basal excretion values (TAN) expressed as mg N-NH_4^+/day/kg LW in several species.

Species	Tan	Weight (g)	Source
Oncorhynchus mykiss	169.0	NR	Nose (1961)
Lepomis macrochirus	346.8	NR	Savitz (1969)
Cyprinus carpio	104.0	200.0	Ogino *et al*. (1973)
Oreochromis mossambicus	128.0	NR	Jauncey (1982)
Ctenopharyngodon idella	140.0	4.2	Carter and Brafield (1992)
Cyprinus carpio	121.0	65.0	Chakraborty *et al.* (1992)
Clarias gariepinus	167.3	32.2	Buttle *et al*. (1995)
Labeo rohita	119.7	3.0	Chakraborty and Chakraborty (1998)
Dicentrarchus labrax	210.0	37.5	Peres y Oliva-Teles (2001)
Oreochromis spp	468.8	100.0	Valbuena and Váquez (2011)
Oreochromis spp	356.3	498.0	Valbuena and Váquez (2011)
Piaractus brachypomus	177.3	46.1	This study
Piaractus brachypomus	128.7	253.3	This study
Piaractus brachypomus	79.2	520.0	This study

Not reported values (NR).

The excretion values obtained in this experiment, lower than those observed in other species could be explained by the feed used. Feeding in the mentioned reports consisted in practical rations, while semi-purified and highly digestible diets (>95%) with a good essential amino acid balance were used in the present study. This could have affected ammonium excretion considering that protein quantity -in terms of AAE balance-, nutritional composition of ingredients, and their digestibility, are the main factors affecting ammonium excretion in fish (Cho and Bureau, 2001; Green and Hardy, 2008; Peres and Oliva-Teles, 2006)**.**

The inverse relation between ammonium excretion and body weight occurs both in fed and fasted fish (Zakes *et al.*, 2006). Such a relationship was observed in this study for fasted and fed cachamas. The lightest animals had the highest excretion values. Similar findings have been reported in several species such as Siberian sturgeon (*Acipenser baeri;* Jatteau, 1997), Japanese flounder (*Paralichthys olivaceus;* Tanaka and Kadowaki, 1995), European bass (*Perca fluviatilis;* Zakes *et al.*, 2003), and *Aphanius iberus (*Oliva-Paterna *et al., 2007)*. The observed reduction in ammonium excretion rate (weight – specific) as body weight increases could result from the low metabolic rate (weight – specific) of heavy fish compared to those that are still growing. This has been partly explained in terms of the physiological changes that take place during fish ontogeny and also be related to muscle development and the variations in the surface/volume coefficient of respiratory organs (Post and Lee, 1996). Knowledge of these relationships could be useful to estimate ammonium loads in the water of fingerling-producing farms and high-density biomass farming.

Ammonium excretion rates in teleost fish are invariably related to protein consumption (Shuenn-Der *et al.*, 2002) and respond to a nictimeral cycle, at least for several of the species studied (Valbuena and Vásquez, 2011; Lerse*et al.*, 2012). According to this, excretion rate increases up to a maximum peak or postprandial pulse from a baseline when fish are fed only once a day (Martínez, 2002). Excretion after feeding white cachama behaves similarly, with a tendency to increase one hour after feeding. The small animals (P1) fed the three protein levels had a tendency for highest excretion peaks between 16 and 24 hours, while between P2 and P3 the pulse appeared before (around hour 10 from sampling). A direct relationship between protein level and number of excretion pulses was also observed. This means that only one postprandial peak appeared for all weights fed 25% CP, and there were two excretion peaks for 30 and 35% CP levels.

At the end of the sampling processes none of the excretion rates reached the values observed in the first sampling after feeding, which means that more than 24 hours are required to reach the initial values. Similar results were obtained with *Colossoma macropomum* (tambaqui) weighing between 17 and 238 g, which had two to five excretion peaks during 24 hours, at hours 4, 8, 14, 20, and 22 (Ismiño-Orbe *et al.*, 2003).On the other hand, only one excretion peak around hour 10, with all values returning to the baseline, was reported for *Sparus aurata* species gilt-head bream weighing between 50 and 150 g and fed to satiety once a day (Martínez, 2002). Similarly, under the same conditions, a pulse appeared about hour 8 and then descended to the initial secretion levels obtained at the beginning of the experiment reported by Dosdat *et al.* (1996) for *Dicentrarchus labrax* (bass), *Scophthalmus maximus* (turbot), *Sparus aurata* (porgy), *Salmo trutta fario* (brown trout) and *Oncorhynchus mykiss* (rainbow trout) of 100 g. In another study with young 70 g *Oncorhyncus mykiss* the excretion pulse appeared between hours 2 and 8, with the maximum peak around hour 4, followed by a return to the initial values (Gelineau *et al.*, 1998). The excretion peak appeared at hour 6 after 79 g young *Dicentrarchus labrax* were fed an extruded diet; with another peak around hour 7.5 after eating a pelleted diet (Ballestrazzi *et al.*, 1998). Burel*et al.* (1996), working with young *Scophthalmus maximus* species, found that duration of postprandial pulses of ammonium excretion depends on the dietary protein increase. Postprandial pulse of European eel (*Anguilla anguilla*, 35 g of average weight) in a 12 hours sampling period started around hour 6 and extended until the end of the sampling (García-Gallego *et al.*, 1999). For sea water species, such as *Lutjanus argentimaculatus* (mangrove snapper) and *Epinephelus areolatus* (areolate grouper), the top peaks at 25 ºC were observed around hours 2 and 4, respectively, and then returned to their initial values (Leung *et al.*, 1999b). A study with *Epinephelus areolatus* obtained similar results: ammonium excretion peaked between hours 2 and 5 after feeding (Leung *et al.*, 1999). For the sea water species *Sciaenops ocellatus* the pulse appeared around hour 4 after feeding (Webb and Gatlin III, 2003).

According to the present results, cachama´s pulses are longer, more irregular, and last during the whole sampling time (26 hours) while the species mentioned above have shorter, regular periods, and tend to return to their initial levels before 24 hours. These differences in postprandial pulse and its occurrence after feeding could depend on the diet used. According to Jobling (1981b), excretion increases with the quantity of oxygen intake and digestible nitrogen. Furthermore, physiological and particular nutritional habits of each species can be influential. According to this, gastric evacuation time in connection with ration size, which also depends on the size of the stomach, could affect the excretion periods. Riche *et al.* (2004) demonstrated that tilapia (*T. nilótica*), which has a relatively small stomach, has a rapid evacuation rate that ends few hours after ingestion. *Piaractus*, the same genus cachamas belong to, are opportunistic with omnivorous habits and have a relatively big stomach (Vásquez-Torres, 2005). Consequently, they are able to ingest high amounts of food in a single meal (their gastric evacuation time is 0.0490 mg/h; González and González, 1996). The same has been demonstrated for other species (Jensen *et al.,* 1987; Leung *et al.*, 1999b). Based on this, we suggest that is the reason for the elongated and irregular pulses lasting 24 or more hours in this experiment.

Considering this results, it can be concluded that the basal excretion and feeding rates for 46.07 g young cachamas fed a semi purified diet were higher with respect to those of bigger animals (253 and 520 g). These data can be used to determine ammonium loads under various systems, their possible impact on water quality parameters, and their effect on fish welfare. White cachamas have a long postprandial pulse that lasted during the 26 hours sampling period, and can have one or two excretion peaks according to the protein level. This finding could suppose feeding habits last above 26 hours. Nevertheless, studies on the subject are necessary to determine growth rates. Excretion in fasted fish increases in an inverse proportion to fish weight. Similarly, when fish were fed each protein level, the highest excretion values corresponded to the lighter animals, which can be explained by their higher metabolic rate.

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