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Ammonia excretion at different life stages of silver catfish

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ABSTRACT. This study examined ammonia excretion at different life stages (eggs, larvae and juveniles) in silver catfish (*Rhamdia quelen)* and determined the influence of fasting time on ammonia excretion. Eggs and larvae were collected from incubators at different times after fecundation and placed in chambers. Juveniles were separated into two weight classes (2-50 g and 150-320 g) and placed in individual chambers after feeding. Water was collected from each chamber to determine ammonia excretion. Ammonia excretion by the eggs was low, but when hatching began approximately 28h after fecundation, excretion increased until 48h after fecundation. In fasting silver catfish juveniles, there was a significant negative correlation between ammonia excretion and weight. Moreover, ammonia excretion decreased significantly after 12 and 48h of fasting (compared to 6h fasting) in the smallest and largest specimens, respectively. Consequently, during incubation of silver catfish eggs, water renovation must be increased at hatching time to avoid a build-up in the concentration of ammonia. In addition, as ammonia excretion in this species increases after feeding, feed must be discontinued when ammonia levels in the tanks are high to avoid a further increase of this metabolite and consequent mortality of silver catfish.

Keywords: eggs, fasting time, nitrogenous compound, larvae.

Excreção de amônia em diferentes estágios de vida do jundiá

RESUMO. Verificou-se a excreção de amônia em diferentes estágios de vida (ovos, larvas e juvenis) e determinou-se a influência do tempo de jejum na excreção de amônia no jundiá, Rhamdia quelen. Ovos e larvas foram coletados das incubadoras em diferentes tempos após a fecundação e colocados em recipientes. Juvenis foram separados em duas classes de peso (2-50 e 150-320 g) e após a alimentação foram colocados em recipientes individuais. As águas foram coletadas de cada recipiente para determinação da excreção de amônia. A excreção de amônia pelos ovos foi baixa, mas iniciada a eclosão – 28h após a fecundação, ela aumentou até as 48h. Nos juvenis em jejum, houve significativa correlação negativa entre a excreção de amônia e o peso. Além disso, a excreção de amônia diminuiu significativamente após 12 e 48h de jejum nos espécimes menores e maiores, respectivamente. Portanto, a renovação da água deve ser aumentada no momento da eclosão dos ovos de jundiá para evitar aumento da concentração de amônia. Como nessa espécie a excreção de amônia aumenta após a alimentação, quando os níveis nos tanques forem elevados a alimentação deve ser suspensa para evitar aumento adicional deste metabólito e mortalidade.

Palavras-chave: ovos, tempo de jejum, composto nitrogenado, larva.

Introduction

Ammonia is the dominant end product of nitrogen metabolism in most teleosts, and is toxic at low concentrations, particularly in NH_3 (unionized ammonia) form (CHEW et al., 2006; FELIPO; BUTTERWORTH, 2002; IP et al., 2004; WICKS; RANDALL, 2002). The main internal source of ammonia in fish is through the catabolism of proteins, and most of this waste product is produced in the liver of fish during the transamination of amino acids followed by the deamination of glutamate (WICKS; RANDALL, 2002). Ammonia is mainly excreted through the gills (WILKIE, 2002), and teleosts usually increase the excretion of ammonia after feeding (ALTINOK; GRIZZLE, 2004).

Compared to the number of studies conducted on adult stages of teleosts, only a small number of studies have examined nitrogen metabolism during the early development of teleosts. Ammonia appears to be the dominant end product during the embryonic and yolk sac stage of freshwater teleosts (DABROWSKI et al., 1984; OLIVA-TELES; KAUSHIK, 1990; WRIGHT et al., 1995). Ammonia excretion in *Caregonus lavaretus* increased during the pre-hatching period, from 52.1 to

163.2 μg h^{-1} 10^3 $eggs^{-1}$ (DABROWSKI et al., 1984), and high rates of ammonia excretion were observed (> 100 and > 50 μg N h^{-1} 10^3 $eggs^{-1}$) in common carp, *Cyprinus carpio*, at hatching and at the onset of free-swimming stages, respectively (KAUSHIK et al., 1982).

Altinok and Grizzle (2004) reported that dietary protein intake is the most important factor affecting ammonia excretion. In fish fed until satiation, ammonia production may be 10 times higher than in starved fish (WOOD, 1993).

The silver catfish, *Rhamdia quelen* (Quoy and Gaimard, 1824; Heptapteridae), is found from southern Mexico to central Argentina; the species is bred more intensively in Brazil, Uruguay and Argentina. Moreover, the species shows two spawning peaks $year^{-1}$ (GOMES et al., 2000); embryological development is fast, and larval development occurs in 3-5 days (AMORIM et al., 2009; PEREIRA et al., 2006). Therefore, the aim of this study was to examine ammonia excretion at different life stages (eggs, larvae and juveniles) and determining the influence of fasting time on ammonia excretion in silver catfish.

Material and methods

Silver catfish eggs (mean weight = 5.0 mg) were obtained after induced spawning. The brood fish received one dose of carp pituitary extract (female = 5 mg kg^{-1}; male = 3 mg kg^{-1}, according to LEGENDRE et al., 1996) and were then extruded after nine hours. The oocyte mass was placed in a plastic container, and milt was then added to provide fertilization. Half a liter of water at the same temperature as the water in which the brood fish were maintained was added to the container to allow egg hydration (10 min.). Twelve minutes later, the dead eggs were manually separated on Petri dishes, and the viable fertilized eggs (determined by the observation of the initial cell division on a microscope) were placed in incubators (4 L polyethylene bottles – 600 eggs per bottle) at a temperature of 24 ± 1.0ºC and a pH of 7.2 ± 0.2 units. Each bottle received continuous aeration with a 20 W air pump (3.2 L $min.^{-1}$ air flow) that also promoted water movement. The incubators were cleaned daily by suction, and, consequently, at least 30% of the water in the bottles was replaced with water previously adjusted to the same conditions of temperature and pH.

Eggs and larvae were collected from the incubators at 0, 12, 24, 36, and 192h after fecundation and placed in continuously aerated 25 mL chambers (ten replications in each time, n=10 for each replication) for 12h. Water samples (5 mL) were taken at the beginning and end of each 12h period and the ammonia concentration was immediately measured. Afterwards, all eggs and larvae from each replication were weighed in a scale (precision ± 0.00001 g). Larvae received commercial diet (32% crude protein) after hatching (approximately 28h after fecundation), in constant intervals (every two hours).

Silver catfish juveniles (2 - 50 g and 150 - 320 g) were bought from a commercial fish farm near the city of Santa Maria, Rio Grande do Sul State, Brazil, and transported to the Fish Physiology laboratory at the UFSM, where they were maintained for four days in continuously aerated 250 L tanks (aerated using two 20 W air pumps; pH 6.7 ± 0.5 units; temperature 23 ± 1.0ºC; and hardness 32.0 ± 1.0 mg $CaCO_3$ L^{-1}). After this acclimation period, the silver catfish were fed and placed in individual chambers (ten fish for each size range) with approximately 20 times their volume in water. Water was collected from each chamber at 0, 6, 12, 24, 36, and 48h after the transfer and the ammonia concentration was immediately measured. Fish were weighed at the end of water collection and were not fed when inside the chambers.

Dissolved oxygen (6.17 ± 0.8 mg L^{-1}) and temperature (21.5 ± 1.5ºC) were monitored with an oxygen meter (model Y5512). The pH levels (7.2 ± 0.2 units) were verified with a DMPH-2 pH meter, while nitrite values (0.3 ± 0.1 mg L^{-1}) were assessed using a colorimetric method (commercial kit), and water alkalinity (40 ± 1.3 mg $CaCO_3$ L^{-1}) and hardness values (36 ± 1.5 mg $CaCO_3$ L^{-1}) were determined according to Boyd and Tucker (1992). Ammonia concentrations in the water samples were measured according to Verdouw et al. (1978), and ammonia excretion (efflux) was calculated according to Gonzalez et al. (1998). These parameters were observed in all experiments (eggs, larvae and juveniles groups).

Data are reported as means ± S.E.M. Homogeneity of variances between groups was tested by Levene's test. Comparisons of ammonia excretion at different times of embryonic development and larviculture and after fasting in juveniles were conducted using one-way ANOVA and Tukey's test (Statistica software v.5.1). The relationship between weight and ammonia excretion in silver catfish juveniles was assessed using Sigma Plot 11.0 software.

Results and discussion

All physicochemical parameters of the water were kept within the range recommended for this species

throughout the experiment (BALDISSEROTTO, 2004).

Ammonia excretion by eggs was low, but when hatching started approximately 28h after fecundation, excretion increased until 48h after fecundation (Figure 1). Larger larvae (192h after fecundation) presented lower values of ammonia excretion. Therefore, the increase observed during the embryonic stage probably reflects the small but growing amount of respiring tissues and also the elimination of metabolites. However, although the egg capsule or chorion is permeable to ammonia (RAHAMAN-NORONHA et al., 1996), elimination of ammonia is slow in the absence of respiratory convection (ROMBOUGH; MOROZ, 1990, 1997) and direct contact with bulk water. The present results regarding silver catfish are in agreement with studies of ammonia excretion of embryos and larvae of other freshwater teleost species (BUCKING; WOOD, 2008; DABROWSKI et al., 1984; KAUSHIK et al., 1982; OLIVA-TELES; KAUSHIK, 1987, 1990; TERJESEN et al., 1997; WRIGHT et al., 1995). Ammonia excretion increased markedly for up to 48h after hatching, but the trend did not continue at the next sampling point. Interestingly, a similar pattern with an apparent lag phase was observed in studies of other freshwater teleost larvae (DABROWSKI et al., 1984; KAUSHIK et al., 1982; OLIVA-TELES; KAUSHIK, 1987; TERJESEN et al., 1997), and it could result from a high amino acid catabolism associated with the process of hatching.

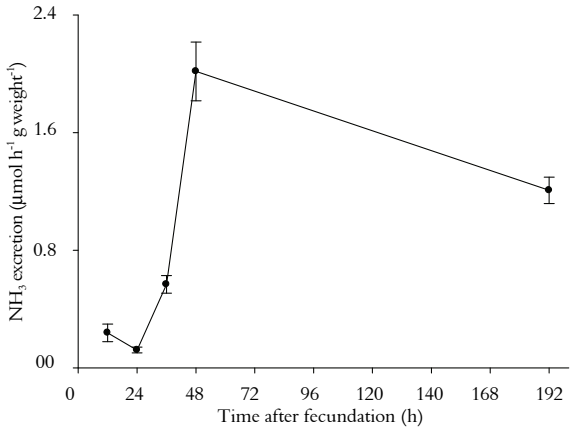

Figure 1. Ammonia excretion in silver catfish eggs and larvae at different times after fecundation.

In fasting silver catfish, there was a significant negative relationship between ammonia excretion and weight after 48h (Figure 2). This is in agreement with higher ammonia urinary excretion associated with lower body mass observed in silver catfish (BOLNER; BALDISSEROTTO, 2007), as well as in walleye, *Sander vitreus* (YAGER; SUMMERFELT, 1993) and tambaqui, *Colossoma macropomum* (ISMIÑO-ORBE et al., 2003). This can be attributed at least partially to ontogenetic changes in the "metabolic intensities" of the different tissues, which tend to decline as fish increase in size (WOOD, 1993).

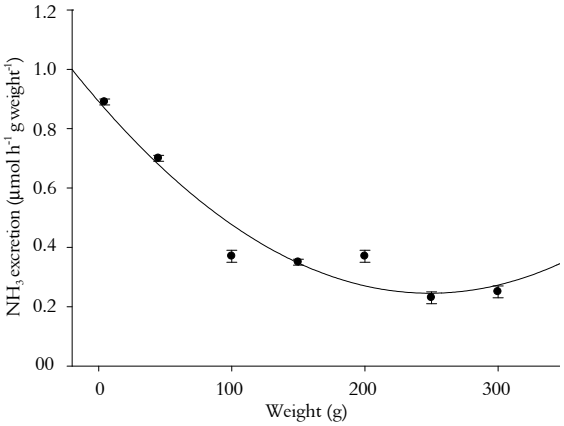

Figure 2. Ammonia excretion in silver catfish as a function of weight. This relationship is described by the following equation: $\hat{y} = -0.976 + 2.163 / (1 + (X / 461.57)^{0.42})$, $r^2 = 0.960$; where $\hat{y}$ = ammonia excretion ($\mu g\ h^{-1}\ g\ weight^{-1}$) and x = fish weight (g).

Ammonia excretion decreased significantly after 12 and 48h of fasting (compared to 6h of fasting) in the smallest and largest specimens, respectively (Figure 3). Ammonia excretion is associated with feeding changes during the postprandial period. Several studies (Dosdat et al. (1996), with five teleost fish species divided into two weight classes, 10 and 100 g; Gelineau et al. (1998), with 70 g rainbow trout; Leung et al. (1999), with 83 g *Epinephelus areolatus*; Bucking and Wood (2008), with 300 - 400 g rainbow trout) have documented that elevated ammonia excretion rates occurred between 2 and 12h after feeding. As reported above, ammonia excretion increases during the postprandial period in fish, suggesting that internal ammonia levels have risen after feeding (WICKS; RANDALL, 2002). Interestingly, ammonia excretion increased again in small silver catfish after 24 - 48h of fasting. As explained earlier, small fish have a higher metabolic rate than larger fish, and it is possible that after 24h fasting they use protein rather than lipid and carbohydrate resources to generate energy. This protein catabolism would lead to an increase in ammonia excretion. In agreement with this hypothesis, rainbow trout fasted for one week presented ammonia excretion levels as high as those observed in trout 6 - 12h after feeding (BUCKING; WOOD, 2008).

Postprandial nitrogenous excretion is known to be influenced by the type of feed (ENGIN;

CARTER, 2001; LAM et al., 2008; WEBB JR.; GATLIN III, 2003) and fish size (JOBLING, 1981; LEUNG et al., 1999), and thus it is essential that future studies examine the intricate relationships between feed types qualities^{-1} and the induction of N retention mechanisms in silver catfish at different stages of development.

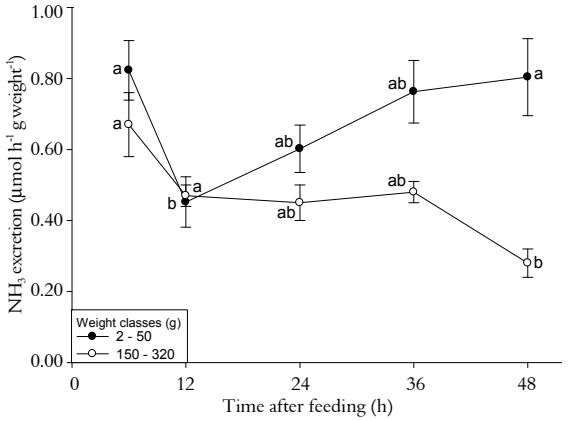

Figure 3. Ammonia excretion in silver catfish of two different weight classes as a function of fasting time. Different letters indicate significant differences between the times after feeding within the same weight class ($p < 0.05$).

Conclusion

In conclusion, during the incubation of silver catfish eggs, water renovation must be increased at hatching time to avoid a build-up of ammonia. Additionally, since ammonia excretion in this species increases after feeding, feeding must be discontinued when ammonia levels in the tanks are high, to avoid a further increase of this metabolite and consequent fish mortality.

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