Aquaculture 306 (2010) 108–115

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The impact of elevated water ammonia concentration on physiology, growth and feed intake of African catfish (*Clarias gariepinus*)

Edward Schram [a,*,1], Jonathan A. C. Roques [a,b,1], Wout Abbink [a], Tom Spanings [b], Pepijn de Vries [a], Stijn Bierman [a], Hans van de Vis [a], Gert Flik [b]

[a] IMARES Wageningen UR, P.O. Box 67, 1970 AB, IJmuiden, The Netherlands
[b] Department of Animal Physiology, Institute for Water and Wetland Research, Faculty of Science, Radboud University Nijmegen, Heyendaalseweg 135, 6525 AJ, Nijmegen, The Netherlands

ARTICLE INFO

Article history:
Received 22 January 2010
Received in revised form 31 May 2010
Accepted 3 June 2010

Keywords:
African catfish
Ammonia
Specific growth rate
Plasma ammonium
Feed intake
Stress physiology
Gill morphology

ABSTRACT

The threshold concentration for NH_3 in rearing water of African catfish (*Clarias gariepinus*) was assessed. African catfish with an initial mean (SD) weight of 141.0 (24) g were exposed to five different T_{amm} [sum of NH_3 and NH_4^+] concentrations: 0.37 (Control), 1.06, 2.12, 5.16 and 19.7 mM, which concurs with NH_3 concentrations of 4 (Control), 14, 38, 176 and 1084 μM. Plasma concentrations of NH_4^+, cortisol, glucose and lactate, plasma osmolality, gill morphology, branchial Na^+/K^+-ATPase activity, feed intake and specific growth rate were monitored. No effect of water NH_3 on plasma NH_4^+ concentrations was detected. Feed intake and specific growth rate were severely affected at exposure to water NH_3 concentrations above 90 μM (calculated EC_{10} values: 89 and 122 μM). No major disturbances in physiological blood parameters were observed at these NH_3 concentrations, but gill morphology (a remarkably sensitive stress indicator) deteriorated significantly. Based on the lower limit of the 95% confidence interval for EC_{10}, we advise for African catfish not to exceed a water NH_3 concentration of 24 μM (0.34 mg NH_3-N/L). This finding is relevant for design and management of African catfish production systems.

1. Introduction

Fish produce nitrogenous wastes through catabolism of amino acids (Wood, 1993). The majority of fresh water and marine teleost fish are ammonioteles and excrete most of their nitrogenous wastes as ammonia across the gills to the water (Wilkie, 2002). The mechanisms involved in branchial ammonia excretion remain controversial. In the most recently proposed model for branchial ammonia excretion, simple NH_3 diffusion down the partial pressure gradient is the predominant mechanism under normal conditions. At high water ammonia concentrations, when NH_3 diffusion is impaired or even reversed, several active NH_4^+ excretion pathways, involving Rhesus (Rh) glycoproteins as membrane transporters, facilitate ammonia efflux (Wright and Wood, 2009).

High water ammonia leads to rapid accumulation of ammonia in plasma and tissues (Wright et al., 2007), where it is mainly present as NH_4^+ at physiological pH (Wilkie, 2002). High internal NH_4^+ causes neurotoxicity (Cooper and Plum, 1987 in Wilkie, 2002).

High water ammonia, caused by high feed loads and high fish densities, is an important limiting factor for intensive aquaculture (Boeuf et al., 1999). Water ammonia should therefore be kept below species-specific threshold levels.

The African catfish (*Clarias gariepinus*) is empirically known to be highly tolerant to ammonia toxicity (Ip et al., 2004a). Several defence strategies allow this fish to cope with increased internal ammonia, for instance during prolonged air exposure or during periods of draught, when the fish survive in mud pools. The defence strategies include active excretion of NH_4^+, reduced ammonia production by reduction of proteolysis and/or reduced amino acid catabolism and a high ammonia tolerance of tissues and cells. Moreover, it appears that this catfish reduces membrane and skin permeability to NH_3 in response to high water ammonia concentrations (Ip et al., 2004a).

The NH_3 threshold concentrations for physiological disturbances, feed intake and growth are unknown for African catfish. As a result it is unclear whether intensive farming of this fish species at high water NH_3 concentrations results in physiological disturbances, reduced feed intake and reduced growth, and thus may impinge on the welfare of the fish. In the present study, African catfish (*Clarias gariepinus*) was exposed to increased water ammonia for 34 days to establish NH_3 threshold concentrations.

2. Materials and methods

2.1. Experimental conditions

African catfish (*Clarias gariepinus*) were obtained from Fleuren-Nooijen BV, Someren, The Netherlands. Fish (n = 168) were randomly divided over 12 30-L rectangular glass tanks and allowed to acclimatise

* Corresponding author.
E-mail address: edward.schram@wur.nl (E. Schram).
[1] Contributed equally to this study.

0044-8486/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2010.06.005

to the experimental conditions for 7 days. At the start of the 34-day experiment, the overall initial mean (SD) individual weight was 141.0 (24) g. The resulting mean stocking density was 65.8 kg/m^3, well below fish densities found at commercial farms for this size class (100 to 300 kg/m^3, Van de Nieuwegiessen et al., 2009). The treatment of the fish was in accordance with Dutch law concerning animal welfare, as tested by the ethical committee for animal experimentation of Wageningen UR Livestock Research (number 2009045.a).

We aimed at a threefold ammonia concentration increase for five consecutive treatments and a concentration range around the highest total ammonia concentrations observed at commercial farms (4.2 to 5.0 mM) without exceeding the acute toxic total ammonia concentration (96 h LC_{50}) of 380 mM (Britz, 1988 in Ip et al., 2004b). Five (1 to 5) different total ammonia [T_{amm} = sum of NH_3 and NH_4^+] concentrations in the rearing water were used: 0.37 (Control), 1.06, 2.12, 5.16 and 19.7 mM. These T_{Amm} concentrations concurred with NH_3 concentrations of 4 (Control), 14, 38, 176 and 1084 μM (Table 1). Treatments were executed in duplicate and assigned randomly to the tanks. Treatments are hereafter referred to as 4, 14, 38, 176 and 1084 μM NH_3.

During the acclimatisation and experimental period, all tanks were supplied with local tap water via a header tank at a flow of 185 L/d for each tank. During the experimental period, experimental ammonia concentrations were realised by infusion of ammonium chloride (NH_4Cl) stock solutions (Table 1). Stock solutions were pumped into the tanks by a peristaltic pump (Watson Marlow 505 S; Rotterdam, The Netherlands) at a flow of 4.75 L/d per tank. Each tank was equipped with an air stone to mix the stock solution with the tank water. Flows were monitored and adjusted as required to reach the experimental ammonia concentrations. Sodium bicarbonate ($NaHCO_3$) was added to the stock solutions to adjust the pH. In addition, sodium chloride (NaCl) was added to the stock solutions to compensate for the differences in chloride concentrations arising from NH_4Cl addition. Total predicted sodium concentrations in the tanks from $NaHCO_3$ and NaCl combined were equal among treatments (Table 1). Fresh stock solutions were prepared daily. The salinity of the tank water resulting from the infusion of stock solutions did not exceed 5 g/L. According to Clay (1977) African catfish tolerate salinities up to 10 g/L.

Water quality (Table 2) was monitored by daily (between 1 and 2 pm) measurements of total ammonia (T_{Amm}) concentrations (photometrically, Hach Lange DR2800), water temperature, pH, dissolved oxygen concentrations (Hach Lange HQ 40 multimeter) and conductivity (WTW Cond 315i) in all individual experimental tanks. NH_3 concentrations were calculated from the temperature, pH and salinity dependent molar fraction of NH_3 and the measured T_{Amm} concentrations (Creswell, 1993). Ammonia concentrations were gradually increased to the designated concentrations during the first four days of the experimental period. Mean water temperature was 27.0 °C throughout the experimental period.

2.2. Plasma sampling

One day before ammonia exposure started (day 0), the fish from two tanks were sampled. After 34 days exposure to ammonia, the fish from the 10 remaining tanks were sampled (two tanks for each of the five treatments, 12 fish per tank). Fish were rapidly caught with a net and quickly anaesthetised in 0.1% (v/v) 2-phenoxyethanol (Sigma, St. Louis, USA). Within two minutes, blood had been taken by puncture of the caudal vein using a lithium heparinised Vacuette blood collection system (Greiner Bio-One GmbH, Kremsmünster, Austria). The blood was centrifuged for 10 min (14,000×*g*, 4 °C) and the plasma obtained was stored at −20 °C.

2.3. Plasma NH_4^+

Plasma NH_4^+ was determined using a commercially available test kit (Instruchemie, Delfzijl, The Netherlands), with a protocol adapted for a 96-well microplate application.

2.4. Plasma cortisol

Cortisol was measured by radioimmunoassay (Metz et al., 2005) with commercially available antiserum (Campro Scientific, Veenendaal, The Netherlands). Samples of 10 μl of 1:5 (v/v) water diluted plasma were incubated overnight at 4 °C with 100 μl first antibody (IgG-F-1; 1:400), 2000 cpm ^{125}I-cortisol (Amersham, Buckinghamshire, UK) and 100 μl secondary antibody (GARGG; 1:160). All constituents were dissolved in cortisol RIA buffer [0.063 M Na_2HPO_4, 0.013 M Na_2EDTA, 0.02% (w/v) NaN_3, 0.1% (w/v) 8-anilino-1-naphthalene sulfonic acid (Sigma) and 0.1% (w/v) bovine gamma globulin (Sigma)]. Immune complexes were precipitated by addition of 1 ml ice-cold 5% (w/v) polyethylene glycol and 2% (w/v) bovine serum albumin (Sigma) and subsequent centrifugation (20 min, 2000×*g*, 4 °C). Pellets were counted in a gamma counter (1272 Clinigamma, LKB Wallac, Turku, Finland).

2.5. Plasma glucose and lactate

Plasma glucose and lactate were measured with commercially available enzymatic test kits (Instruchemie, Delfzijl, The Netherlands), with protocols adapted to a 96-wells microplate. For glucose, 10 μl sample or standard (5.55 mM glucose) was mixed with 200 μl reagent and incubated for 10 min at 25 °C. Absorbance was read within 60 min at 495 nm. For lactate, 10 μl sample or standard (4.44 mM lactate) or blank (8% perchloric acid) was mixed with 290 μl of lactate reagent and incubated for 20 min at 37 °C. Absorbance was read at 355 nm.

2.6. Osmolality

Plasma osmolality (sample volumes: 50 μl) was measured with a cryoscopic osmometer (Osmomat 030, Gonotec, Germany). Deionized water (0 mOsmol/kg) and a standard solution (300 mOsmol/kg) were used as reference.

2.7. Gill morphology

One gill arch was removed immediately after blood sampling and placed overnight in Bouin's fixative (75 ml saturated picric acid, 25 ml saturated formaldehyde, 5 ml acetic acid). Gill sections were made to include the trailing edge of the filament where the chloride cells

Table 1
Compositions of the daily prepared treatment specific stock solutions and the calculated[a] TAN, sodium and chloride concentrations in the tanks for all treatments.

Treatment	NH_4Cl (g/10 L)	$NaHCO_3$ (g/10 L)	NaCl (g/10 L)	Total Cl^- dose (g/10 L)	Total Na^+ dose (g/10 L)	Predicted tank [Na^+] (g/L)	Predicted tank [Cl^-] (g/L)
1	0	0	1555	933	622	1.6	2.4
2	15	9	1549	939	622	1.6	2.4
3	45	47	1523	943	622	1.6	2.4
4	135	180	1432	948	621	1.6	2.5
5	404	530	1192	982	620	1.6	2.5

[a] Based on equal flow rates per tank of 4.75 L/day for the stock solutions and 185 L/day for the tap water flow.