



Changes in intestinal morphology and amino acid catabolism in common carp at mildly elevated temperature as affected by dietary mannanoligosaccharides

F. Geda^{a,b,f,*}, A. Rekecki^e, A. Decostere^e, P. Bossier^b, B. Wuyts^c,
I.D. Kalmar^d, G.P.J. Janssens^a

^a Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium

^b Laboratory of Aquaculture and Artemia Reference Centre, Faculty of Bioscience Engineering, Ghent University, Rozier 44, B-9000 Ghent, Belgium

^c Department of Clinical Chemistry, University Hospital Ghent, De Pintelaan 185, B-9000 Ghent, Belgium

^d Department of Molecular Biotechnology, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, B-9000 Ghent, Belgium

^e Department of Morphology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

^f Department of Animal Sciences, College of Agriculture and Veterinary Medicine, Jimma University, P.O. Box 307, Jimma, Ethiopia

ARTICLE INFO

Article history:

Received 24 May 2012

Received in revised form

21 September 2012

Accepted 22 September 2012

Keywords:

Common carp

Elevated temperature

Mannanoligosaccharide

Gut morphology

Amino acid

Acylcarnitine

ABSTRACT

A study was conducted to evaluate whether dietary mannanoligosaccharides (MOS) modulate the effect of mildly elevated water temperature on intestinal morphology and some selected whole blood amino acids and acylcarnitines in common carp (*Cyprinus carpio carpio*). Twenty fish in 10 aquaria were randomly assigned to either a control diet or the same diet with 4g/kg MOS. A 35-day period at ideal temperature (23.2 °C) was followed by 14 days at elevated temperature (26.1 °C). After both temperature periods, one fish per aquarium was euthanized for the intestinal morphology and whole blood analysis on free amino acids and carnitine esters. The elevated temperature period decreased the number of neutral (P=0.031) and acid (P=0.004) mucin-secreting goblet cells and tended to reduce (P=0.056) the fold height in the midgut, irrespective of MOS supplementation. In both periods, supplementation of MOS increased (P=0.035) the number of goblet cells in the hindgut, but other histomorphometries were unaffected. Free concentrations of whole blood amino acids were increased after the mildly elevated temperature period: valine (P=0.002), leucine (P=0.001), methionine (P<0.001), phenylalanine (P=0.001) and tyrosine (P=0.001), but not affected by MOS supplementation. None of the carnitine esters were altered by the elevated temperature except propionyl carnitine that was higher (P=0.003) after the mildly elevated temperature of 14 days. Supplementation of MOS only tended to reduce the tiglyl carnitine (P=0.069) and methylmalonyl carnitine (P=0.078) concentrations. The analysis of free amino acids and carnitine esters could not support the hypothesis that MOS counteracts depressing effects of elevated temperature on amino acid catabolism. In conclusion, moderate elevation of water temperature could lead to considerable changes in gut histology and metabolism.

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Abbreviations: AB-PAS, alcian blue-periodic acid-Schiff; ATP, adenosine triphosphate; CoA, co-enzyme A; DIT, diet-induced thermogenesis; HE, hematoxylin and eosin; MOS, mannanoligosaccharides.

* Corresponding author at: Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Heidestraat 19, B-9820 Merelbeke, Belgium. Tel.: +32 488 902 552; fax: +32 0 9 264 78 48.

E-mail address: fikremariamgeda.ararame@ugent.be (F. Geda).

1. Introduction

The consumption of aquatic products keeps increasing because of their importance as dietary protein source and high contents of health-associated fatty acids such as docosahexaenoic acid and eicosapentaenoic acid, and other nutrients that are considered healthy (Liao, 2009). However, global climate change was suggested to potentially affect aquaculture sector in warmer climate developing countries by lowering productivity in wild fish populations and in intensive aquaculture systems worldwide (Ficke et al., 2007). Rising water temperature up to a certain limit may favor aquaculture production by increasing growth rate and reducing the maturation period of fish (Chatterjee et al., 2004; Debnath et al., 2006). On the other hand, several studies indicated that a change in water temperature beyond optimum limits of a particular fish species influences the body temperature, oxygen demand, health, feed utilization and growth performance (Linthicum and Carey, 1972; Houlihan et al., 1993; Azevedo et al., 1998; Wedemeyer et al., 1999; Chatterjee et al., 2004; Debnath et al., 2006).

Unlimited feed supply under elevated water temperature reduces feed intake in fish and gut absorption capacity due to a decrease in surface area of microvilli, both resulting in depressed growth performance (Jobling, 1994; Garriga et al., 2005; Song et al., 2010). A study in broiler chickens has shown that fermentable oligosaccharides alter gut morphology with some concomitant changes in oxidative stress (De Los Santos et al., 2005). A study by Dimitroglou et al. (2008) under normal temperature reported that MOS increased the absorptive surface area and microvilli density and length in rainbow trout. Yet, a study in pigeons under thermoneutral environment concluded that dietary MOS reduced the intestinal morphology (villus length, crypt depth, muscularis thickness) because of reduced bacterial challenge (Abd El-Khalek et al., *in press*). Furthermore, even in species with a relatively undersized large bowel like the cat, prebiotic-induced changes in the intestinal environment can limit amino acid catabolism, thus promoting the use of lipids and carbohydrates as energy source (Verbrugghe et al., 2009). The concomitant drop in heat production per gained adenosine triphosphate (ATP) can especially be helpful under heat stress conditions. The potential of dietary MOS to support intestinal morphology and reduce amino acid catabolism has so far not been studied in fish under elevated temperature conditions. Therefore, we investigated the effect of dietary MOS supplementation on gut morphology and some selected whole blood amino acids and acylcarnitines during mildly elevated temperature in common carp.

2. Materials and methods

2.1. Experimental animals and design

Twenty common carp (*Cyprinus carpio carpio*) fingerlings (average body weight of 45.1 ± 1.4 g) were obtained from Wageningen University Animal Sciences, Zodiac, Wageningen, The Netherlands. The fish were transported in a double polyethylene bag with sufficient aeration to the Laboratory of Animal Nutrition, Faculty of Veterinary Medicine, Ghent University, Belgium. All the experimental fish were acclimated to the laboratory conditions on a common diet for 14 days and randomly allocated per two in ten 63 L-glass aquaria of 60 cm × 30 cm × 36 cm (JUWEL Aquarium, Rotenburg, Germany) of recirculating tap water with continuous aeration through a biological filter. Following the acclimation period, the fish were fasted for 24 h, weighed (average initial body weight of 48.2 ± 1.9 g) and stratified into the ten aquaria. The aquaria were randomly assigned to two treatment groups, control ("Control") and MOS ("MOS"), each group with five aquaria with two fish each. The experimentation, housing and ethical procedures carried out in this experiment have been approved by the Ethical Committee of Laboratory Animals, Faculty of Veterinary Medicine, Ghent University, Belgium.

2.2. Feed, feeding protocol and elevated temperature

The control group was fed a complete carp diet, S9029-S012/-S015 (ssniff Spezialdiäten GmbH, Soest, Germany) (Table 1) and the MOS group was given the same control diet supplemented using hand spray (AVEVE Group, Leuven, Belgium) with 4 g/kg yeast-derived MOS (Alltech, Dunboyne, Ireland) at a feeding rate of 1.5% of body weight per day. The two experimental diets were provided at normal temperature of 23.2 °C for 35 days. At the end of the 35-day feeding period, each fish was fasted for 24 h and the elevated temperature experiment was conducted on individual fish, those not sampled for normal temperature analysis. The water temperature in each aquarium was increased from 23.2 °C to 26.1 °C at a rate of 3 °C/36 h. The elevated temperature was applied for two weeks. Feeding occurred at 09:00 and 17:00 and the feeding regime remained the same throughout the study. All aquaria were maintained at 12:12 h light–dark photoperiod with fluorescent lights controlled by timers.

2.3. Water quality parameters

At the temperature of 23.2 °C, daily pooled average pH (7.0, 7.0) (Merck KGaA, Darmstadt, Germany), dissolved oxygen (6.52, 6.54 mg/L) (Hanna Instruments Srl, Nufalau, Romania) and two-week intervals ammonium (<0.05, <0.05 mg/L) and nitrite (0.10, 0.09 mg/L) (JBL GmbH and Co KG, Neuhofen/Pfalz, Germany) were measured for the control and MOS groups, respectively. Similarly, at the elevated temperature of 26.1 °C, the daily pooled average pH (7.6, 7.6), dissolved oxygen

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