# IMPACT OF DIETARY ELECTROLYTE BALANCE (DEB) AND DISSOLVED O2 (DO) LEVEL ON FEED INTAKE, GROWTH AND O2 CONSUMPTION IN RAINBOW TROUT (ONCORHYNCHUS MYKISS)

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#### Introduction

Dietary and environmental conditions may deeply alter acid-base balance. Acidbase homeostasis disturbances appear to increase the  $O_2$  consumption required for maintenance metabolism, as fish needs several energy consuming processes essential to keep this balance. Acid-base homeostasis can be affected by DEB which in turn will alter the maintenance energy expenditure [1, 2]. DEB is defined as the sum of the mineral cations minus the sum of mineral anions present in the diet. A low DEB diet has acidic property, while high DEB diet has alkaline property. Alterations in DEB can trigger mechanisms to counteract acid-base imbalances, affecting as well the efficiency of the digestive process. Feed intake decreases with decreasing DO, as this physiological trait is limited by the  $O_2$  uptake capacity in fish. We investigated changes on feed intake, growth performance,  $O_2$  consumption, nutrient digestibility, nitrogen and energy balance, together with several metabolic markers in an isogenic heterozygous family of rainbow trout subjected to a combination of nutritional (DEB, high vs. low) and environmental (hypoxia vs. normoxia) long term challenges (42 days).

## Material and Methods

An isogenic trout line was employed in this study (produced by GABI/La Peima, INRA, France). Fish were housed in 12 tanks (Aquatic Metabolic Unit, WU, The Netherlands) according to a  $2 \times 2$  design. Tanks (200 L) were divided into 3 blocks, with each treatment in triplicates ( $14 \pm 1^{\circ}$ C, 12L:12D, fresh water,  $10.2 \pm 0.2 \text{ mg O}_2 \text{ L}^{-1}$ ). The oxygenation unit maintained the DO level by injecting  $O_2$  and using automatic probes for the detection of water flow and  $O_2$  consumption. The unit was also equipped with faecal collectors for measuring digestibility. Trout were weighed (115  $\pm$  2 g, mean  $\pm$  SD) and 30 fish were randomly assigned to each tank. Two isoproteic (45% DM) and isoenergetic (22 kJ gDM<sup>-1</sup>) diets were extruded as floating 4 mm pellet with 0.01% yttrium oxide addition for digestibility determination. The diets were formulated to provide a contrast in DEB by adding Na<sub>2</sub>CO<sub>3</sub> and diamol, resulting in electrolyte balanced or imbalanced diets (DEB 200 or DEB 700 mEq Kg<sup>-1</sup>, respectively). Fish were fed to apparent satiation the assigned diets twice a day for 42 days. Feed intake was quantified on daily basis and faeces were collected [3]. Difference in DO level was induced by adjusting the water flow into the tanks [4]. For normoxic tanks, DO level in the outflowing water remained above  $7.0 \text{ O}_2 \text{ L}^-$ <sup>1</sup>. DO levels in the outflowing water remained at  $4.0 \pm 0.0 \text{ O}_2 \text{ L}^{-1}$  in all the hypoxic tanks. Fish O<sub>2</sub> consumption was assessed automatically in each tank (every 5 min). A 48-h continuous measurement of total ammonia nitrogen was carried out (automatic sampling in each tank every 3 min). Fish were sampled at the start and at the end of the trial (n=9 per tank) to determine body composition, fish performance and physiological parameters in liver, blood and gastric content (2 or 6 h after feeding). Parameters were analysed for the effects of the diet and DO level along with their interactions by a two-way ANOVA analysis. Data set was tested for normality and for equal variance, and if these assumptions were not met, data transformation was applied. Significant differences were considered for *P*<0.05.

#### Results

Feed intake expressed per unit metabolic body weight ( $FI_{MBW}$ ), growth rate based on metabolic body weight ( $GR_{MBW}$ ) or O<sub>2</sub> consumption (OC) were not affected by the diet, but were altered by DO level (P<0.01) (Figure 1). In particular,  $FI_{MBW}$  (6.7%) and  $GR_{MBW}$ (1.2%) were higher in normoxic compared to hypoxic groups. Also, OC was significantly higher in normoxia than in hypoxia (24%). Contrary, feed conversion ratio (FCR) was not altered by DO level, but was 16% and 4.6% respectively higher, in fish fed the DEB 700 diet (P<0.05). No interactions between DEB and DO level were observed.



**Fig. 1.** Effect of DEB and DO level on (A) feed intake expressed per unit metabolic body weight (A), growth rate based on metabolic body weight (B), feed conversion ratio (C), and O<sub>2</sub> consumption (D) of rainbow trout. Values are mean  $\pm$  SEM (n=3). Different letters indicate differences among treatments ( $\alpha = 0.050$ ).

Diet had a clear effect on blood pH, both in the heart and caudal region (Figure 2). The pH was significantly lower in heart (P<0.01) and caudal region (P<0.05) in fish fed a more alkaline diet (DEB 700). Conversely, the chyme pH was significantly higher in fish fed the DEB 700 diet (P<0.001). The chyme dry matter was lower in fish fed DEB 700 (P<0.001) when compared with DEB 200. DO level had a significant effect on blood pH. Stomach chyme pH was significantly affected by the interaction between diet post-prandial time (P<0.001). Blood pH, stomach chyme DM and pH decreased with time after feeding (P<0.001).



**Fig. 2.** Effect of DEB and DO level on heart blood pH (A), caudal blood pH (B), chyme pH (C), and dry matter content (D) in the stomach of rainbow trout 2 or 6 h after feeding. Values are mean  $\pm$  SEM (n=9). Different letters indicate differences among treatments, while the asterisk indicates differences between sampling times ( $\alpha$ = 0.050). **Discussion** 

Feed intake appear to be unaltered by DEB, but it is decreased by chronic hypoxia in trout. DEB affect acid-base balance, although the  $O_2$  consumption remains unaffected. Stomach chyme pH was higher in fish fed the DEB 700 diet, reflecting in lower blood pH in heart and caudal region as the digestion proceed. Fish fed the DEB 700 diet responded with a reduced alkaline tide, suggesting effective compensatory mechanisms to reduce the impact of the acid-base imbalance. However, increased energy expenditure toward acid-base regulation caused by the DEB 700 diet did not altered growth. This could due to slight differences in feed intake between both diets, undetected on the present study.

### Aknowledgments

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