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Dietary electrolyte balance affects growth performance, amylase activity and metabolic response in the meagre (*Argyrosomus regius*)

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ABSTRACT

Dietary ion content is known to alter the acid-base balance in freshwater fish. The current study investigated the metabolic impact of acid-base disturbances produced by differences in dietary electrolyte balance (DEB) in the meagre (*Argyrosomus regius*), an euryhaline species. Changes in fish performance, gastric chyme characteristics, pH and ion concentrations in the bloodstream, digestive enzyme activities and metabolic rates were analyzed in meagre fed ad libitum two experimental diets (DEB 200 or DEB 700 mEq/kg) differing in the Na_2CO_3 content for 69 days. Fish fed the DEB 200 diet had 60–66% better growth performance than the DEB 700 group. Meagre consuming the DEB 200 diet were 90–96% more efficient than fish fed the DEB 700 diet at allocating energy from feed into somatic growth. The pH values in blood were significantly lower in the DEB 700 group 2 h after feeding when compared to DEB 200, indicating that acid-base balance in meagre was affected by electrolyte balance in diet. Osmolality, and Na^+ and K^+ concentrations in plasma did not vary with the dietary treatment. Gastric chyme in the DEB 700 group had higher pH values, dry matter, protein and energy contents, but lower lipid content than in the DEB 200 group. Twenty-four hours after feeding, amylase activity was higher in the gastrointestinal tract of DEB 700 group when compared to the DEB 200 group. DEB 700 group had lower routine metabolic (RMR) and standard metabolic (SMR) rates, indicating a decrease in maintenance energy expenditure 48 h after feeding the alkaline diet. The current study demonstrates that feeding meagre with an alkaline diet not only causes acid-base imbalance, but also negatively affects digestion and possibly nutrient assimilation, resulting in decreased growth performance.

1. Introduction

Acid-base homeostasis (pH) is essential to maintain physiological activities in fish. However, changes in environmental conditions, including water salinity (Genz et al., 2008; Gilmour et al., 2012), pH (Janssen and Randall, 1975; Wilson et al., 1999), and swimming activity (Wood, 1991; Wood et al., 1983) may disrupt this balance. Despite these challenges, the acid-base homeostasis is maintained within strict limits by several mechanisms, including branchial compensation by ion fluxes, requiring ATPase ion transporters (Claiborne et al., 2002; Evans et al., 2005).

Several studies have demonstrated that feeding has a significant influence on acid-base homeostasis in fish (Bucking et al., 2009; Cooper and Wilson, 2008; Taylor and Grosell, 2006; Wood et al., 2010). In addition, diet composition appears to be an important but less studied factor affecting acid-base balance. Dietary electrolyte balance (DEB) affects the acid-base homeostasis, at least in freshwater species (Chiu et al., 1984, 1988; Dersjant-Li et al., 1999, 2001; Saravanan et al., 2013). The DEB is defined as the sum of the mineral cations minus the sum of mineral anions present in the diet. Differences in DEB may occur when feeding ingredients containing variable quantities of cations (Na, K, Ca and Mg)

Abbreviations: AMS, aerobic metabolic scope; BW, body weight; DEB, dietary electrolyte balance; DM, dry matter; FCR, feed conversion ratio; FI, feed intake; GE, gross energy; GEI, gross energy intake; GIT, gastro-intestinal tract; HSI, hepatosomatic index; MMR, maximal metabolic rate; NFE, nitrogen-free extract; RFRm, relative feeding rate on metabolic weight; RGRm, relative growth rate on metabolic weight; RMR, routine metabolic rate; SMR, standard metabolic rate; SGR, specific growth rate.

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and anions (Cl and P) in their composition are included in the diet, which may occur when commercial feeds are manufactured (Patience et al., 1987). Low DEB value is linked to acidic properties, while high DEB values are related to diets taking on alkaline characteristics.

Previous work in rainbow trout (*Oncorhynchus mykiss*) has shown that DEB has an influence on blood pH, feed intake and ultimately growth (Chiu et al., 1984, 1988). Other freshwater species, such as Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) were significantly affected by DEB as well, displaying changes in energy balance and growth, suggesting the importance of appraising optimal ion levels in fish diets (Dersjant-Li et al., 2001; Saravanan et al., 2013).

Alterations in DEB can trigger mechanisms to counteract an acid-base imbalance, such as gastro-intestinal tract (GIT) acid-base secretion and gill base secretion. These processes operate to reestablish acid-base homeostasis at the expense of an extra energy cost, which could otherwise be allocated to somatic growth (Perez and Munch, 2015). Therefore, acid-base homeostasis may impact fish fitness and growth (Boeuf, 1993; Bœuf and Payan, 2001; Wood and Marshall, 1994).

The digestive tract of marine teleosts has a dual role as a food processing organ and as an osmoregulatory organ (Taylor and Grosell, 2006). Changes in the digestive capacity have been shown to be linked to differences in diet composition (Nikolopoulou et al., 2011) or variations in the environmental salinity (Moutou et al., 2004; Psochiou et al., 2007; Usher et al., 1990) in several fish species. In the case of diets containing different electrolyte content is speculated that the balance between mineral cations and anions can modify nutrient digestibility by changes in the characteristics of the chyme (e.g. pH and viscosity), affecting digestive enzyme activities in the GIT (Patience et al., 1987). However, limited data is available on the impact of DEB on feed consumption, digestive physiology and metabolic response in freshwater fish, while no information is available for euryhaline species, in spite of the overlaid roles of the GIT in water absorption and nutrient digestion/assimilation.

Meagre *Argyrosomus regius* (Asso, 1801), a carnivorous teleost fish, is widely distributed in the North-Atlantic Ocean and Mediterranean Sea (Champagnat and Domain, 1978; Quero and Vayne, 1987). Meagre is an euryhaline species, displaying seasonal reproductive migration to estuaries and salt marshes in natural environments, tolerating salinities between 5 and 42‰ (Cárdenas, 2012; González-Quirós et al., 2011). Due to its excellent flesh quality, meagre has been increasingly produced as selected species for aquaculture diversification (Poli et al., 2003; Quéméner et al., 2002). However, meagre in aquaculture production has shown variable growth rates (Duncan et al., 2013).

Several aspects of meagre physiology remain to be elucidated, in particular the impact of optimized dietary formulation required to maximize growth. Therefore, the objective of the present study was to investigate the effect that an electrolyte-balanced diet (DEB 200) or a rather electrolyte-imbalanced diet (DEB 700) has on growth performance, feed intake, nutrient utilization, digestive enzyme activities, and energy use in the meagre. For this purpose, fish were fed DEB 200 or DEB 700 diets for 69 days to satiation and changes in fish performances were assessed. In addition, fish fed the two diets were sampled to evaluate the effect of DEB on metabolic parameters and ion concentrations in the bloodstream and gastric chyme pH, digestive enzyme activities, and metabolic rates.

2. Materials and methods

All procedures were conducted under the supervision of an accredited expert in laboratory animal science by the Portuguese Veterinary Authority (1005/92, DGV-Portugal, following FELASA category C recommendations), according to the guidelines on the protection of animals used for scientific purposes from the European directive 2010/63/UE. The experiment took place at the Abel Salazar Biomedical Sciences Institute (ICBAS), University of Porto (Portugal). This study was approved by the ORBEA (Organismo Responsável pelo Bem-Estar dos Animais), the Institutional Animal Care and Use Committee (IACUC) of ICBAS.

3. Growth trial

Meagre juveniles were obtained from a commercial fish farm (MARESA, Spain) and transported to ICBAS. Fish were distributed into 6 tanks of 80 L^{-1} capacity with a stock density of 9.75 g L^{-1} and kept during 20 days to adapt to the experimental conditions. The tanks were connected to a closed recirculating system, with a flow rate set for 90 mL L^{-1} in each tank. Temperature (21.5 ± 1.0 °C), salinity (29.0 ± 1.0‰), pH (7.9 ± 0.1), oxygen content (≥95% air saturation), ammonia (≤0.5 mg L^{-1}) and nitrites (≤3 mg L^{-1}) were monitored twice weekly, ensuring constant and suitable conditions (Tucker, 1998). During the acclimatization period (20 days), all fish were fed the control diet (DEB200) at 1.5% body weight/day. The formulation and chemical composition of the control diet, including the electrolyte balance, are similar to commercial feed for juvenile meagre. The use of DEB 700 as the dietary electrolyte level was based on literature data (Saravanan et al., 2013; Tacon and De Silva, 1983). Fish were maintained on a 12 h photoperiod. Each tank containing 20 fish (experimental triplicates) was randomly assigned to the dietary treatments (initial BW 39.4 ± 0.5 g), feeding fish an electrolyte-balanced diet (DEB 200) or an electrolyte-imbalanced diet (DEB 700). The growth trial lasted 69 days, and during that time fish were hand-fed to apparent satiety twice daily (09.00 and 16.00 h). The daily amount of feed ration was registered and used for the feed intake calculation, which was corrected for fish mortality. At the end of the growth trial all fish were sampled to record BW.

4. Diets

The two isoproteic (450 g kg DM^{-1}) and isoenergetic (22 kJ gDM^{-1}) experimental diets (Tables 1 and 2) were produced by Research Diet Services (Wijk bij Duurstede, The Netherlands). The diet consisted of floating 3 mm pellets, designed to give a contrast in electrolyte-content

Table 1
Ingredients of the experimental diets.

Test ingredients (%)	Diets	
	DEB 200	DEB 700
Na_2CO_3	0.3	2.9
Diamol[a]	2.7	0.1
Wheat	27.2	27.2
Wheat gluten	13.0	13.0
Fish meal[b]	13.0	13.0
Fish oil[d]	14.0	14.0
Soya protein concentrate	13.0	13.0
Pea protein concentrate	13.0	13.0
Lysine HCL	0.3	0.3
DL-methionine	0.5	0.5
Monocalcium phosphate	1.5	1.5
$CaCO_3$ (krijt)	0.5	0.5
Vitamin-mineral premix[c]	1.0	1.0
Total	100.0	100.0

DEB, dietary electrolyte balance (mEq/kg). Mineral premix composition (to supply, mg/kg feed): 50, Fe (as $FeSO_4 \cdot 7H_2O$); 30, Zn (as $ZnSO_4 \cdot 7H_2O$); 0.1, Co (as $CoSO_4 \cdot 7H_2O$); 10, Cu (as $CuSO_4 \cdot 5H_2O$); 0.5, Se (as Na2SeO3); 20, Mn (as $MnSO_4 \cdot 4H_2O$); 500, Mg (as $MgSO_4 \cdot 7H_2O$); 1, Cr (as $CrCl_3 \cdot 6H_2O$); 2, I (as $CalO_3 \cdot 6H_2O$).

[a] Diamol GM; Franz Bertram.

[b] RE > 680.

[c] Vitamin premix composition (to supply, mg/kg feed): 10, B1; 10, B2; 20, B3; 40, B5; 10, B6; 0.2, biotin; 2, folic acid; 0.015, B12; 2000, choline (as choline chloride); 100, C (as ascorbic acid C phosphate); 3000 IU, A (as A palmitate), 2400 IU, cholecalciferol (Rovimixw D3–500; DSM, Inc.); 100 IU, E; 10, menadione (as menadione sodium bisulfite, 51%); 400, inositol; 100, antioxidant BHT (E300–321); 1000, calcium propionate. [d]999 Fish oil; Triple Nine Fish protein.

[d] 999 Fish oil; Triple Nine Fish protein.

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Table 2
Proximate composition of the experimental diets.

Parameter	Diets	
	DEB 200	DEB 700
DM (g kg^{-1})	930.3 ± 1.2	926.4 ± 0.3
Ash (g kg DM^{-1})	112.2 ± 0.5	106.5 ± 12.4
Crude protein (g kg DM^{-1})	450.2 ± 1.3	452.3 ± 3.1
Crude fat (g kg DM^{-1})	153.4 ± 0.4	159.5 ± 1.8
NFE (g kg DM^{-1})	315.7 ± 1.7	315.2 ± 1.1
GE (kJ g DM^{-1})	22.0 ± 0.1	22.4 ± 0.2

DEB, dietary electrolyte balance; DM, dry matter; NFE, nitrogen-free extract calculated as follows: NFE = 1000 − (protein + fat + ash); GE, gross energy. Data presented are mean ± standard error of the mean ($n = 3$). No statistical differences were found for any of parameters analyzed between the diets ($P > 0.05$).

(DEB); 200 versus 700 mEq kg^{-1}. This difference was created by adding different amounts of Na_2CO_3 and diamol (inert filler) in the diets.

5. Respirometry

The measurements of oxygen consumption were carried out in individual static respirometers (0.99 L each) submerged in aerated water (≥95% air saturation). A temperature controlling instrument (TMP-REG; Loligo Systems; Tjele, Denmark) was used to maintain water temperature at 21.5 ± 0.1 °C, with similar conditions to the holding tanks. Oxygen partial pressure (kPa) inside the respirometers was measured using fiber optic sensor technology (PreSens, Regensburg, Germany). Data acquisition and MO_2 calculation from the measurements of oxygen content inside the respirometers were carry out with the software AutoResp (Loligo Systems Aps, Tjele, Denmark).

At the end of the growth trial, meagre were fasted 24 h before measurements. A standard chasing protocol (3 min) was then used to assess maximum metabolic rate (MMR) in eight fish from each dietary treatment. Following exhaustion and a 1 min air exposure, each fish was transferred to a respirometer and rates of oxygen consumption (MO_2; mg O_2 kg^{-1} h^{-1}) were recorded immediately by intermittent flow respirometry using established protocols (Genz et al., 2013; Peixoto et al., 2016; Rosewarne et al., 2016). MMR was the highest of three consecutive measures of MO_2. Fish were left undisturbed in the respirometers for 24 h and MO_2 values were logged continuously. Standard metabolic rate (SMR) was estimated as the average of the 10% lowest MO_2 values (Baktoft et al., 2016; Peixoto et al., 2016). Aerobic metabolic scope (AMS) was calculated as the difference between MMR and SMR (in mg O_2 kg^{-1} h^{-1}). Routine metabolic rate (RMR) was estimated as the average MO_2 during the last 4 h of respirometer confinement (i.e. between 09:00 and 13:00 h the days after fish were transferred to the respirometers). Values of MO_2 for individual fish were adjusted to a common body mass of 64 g (study average) using a mass exponent of 0.8.

6. Stomach chyme and plasma analysis

After the growth trial, feed intake was set to 1.5% (DM) of the average body weight per day for 4 weeks. At the end of this period, 2 fish per tank were sampled 2 h after feeding ($n = 6$). Fish were anesthetized using MS-222 (3-aminobenzoic acid ethyl ester, 0,1 g L^{-1}), buffered with $NaHCO_3$ (0.2 g L^{-1}). Blood was extracted from the caudal region in a 1 mL^{-1} heparinized syringe, which was immediately used to measure the blood pH with a pH-meter (Hanna Instruments, USA) following the procedure described by Saravanan et al. (2013). Plasma was obtained after 5 min centrifugation at 10,000 rpm^{-1} and samples obtained were aliquoted and stored −20 °C for later analysis. Fish were euthanized by slicing the cervical spine. The stomach was clamped to avoid content losses and dissected free from other portions of the GIT. The gastric chyme was emptied into a tray, weighed and pH was quantified immediately with a pH-meter (Hanna Instruments, USA) following the procedure described by Saravanan et al. (2013). The chyme samples were dried at 103 °C for 15 h, cooled down in a desiccator for 1 h, the dry weight was determined, and then stored at −20 °C for compositional analysis.

Osmolality in plasma samples was measured in an automatic osmometer (model Type 15, Löser, Germany) according to manufacturer's instructions. The Na^+, K^+,and Ca^{2+} concentrations in plasma samples were analyzed by atomic absorption spectrometry (Pye Unicam SP9) following the procedure described by Wilson et al. (2007). Plasma Cl^- concentration was measured by ion chromatography (Dionex DX-120). Plasma glucose and triglyceride levels were quantified with Glucose RTU and Triglyceride LQ kits, respectively (SPINREACT, Sant Esteve de Bas, Spain), according to manufacturer's instructions.

7. Digestive enzyme activities

Following a similar experimental setup as described in the previous section, 4 fish per tank were sampled 24 h after feeding ($n = 12$). The intestinal tract, including pyloric ceca, was quickly dissected free from adherent adipose tissue and immediately frozen in liquid nitrogen and stored at −80 °C. Digestive enzymes activities were measured in whole intestine, homogenizing the tissue in 5 volumes of buffer solution made by 50 mM Tris-HCl and 200 mM NaCl (dilution 1:5, *w/v*) at pH 8.0 (Rungruangsak-Torrissen, 2007). Enzymatic activities were measured in a microplate reader (Synergy HT, Biotek, USA) under assay conditions to render the highest activity possible and using appropriate controls. α-Amylase activity was quantified by measuring the decrease in 3,5-dinitrosalicylic acid produced by reducing groups released from soluble starch (Bernfeld, 1951). Enzyme activity was calculated as μmol of maltose released per min, monitored at 37 °C, pH 7.4 and 540 nm. Lipase activity was quantified by the enzymatic release of p-nitrophenol from *p*-nitrophenyl palmitate (Winkler and Stuckmann, 1979), whereas activity was calculated as nmol of p-nitrophenol produced per min, monitored at 30 °C, pH 8.0 and 410 nm. Trypsin activity was determined by adding 1 mM of Nα-Benzoyl-l-arginine 4-nitroanilide hydrochloride (BAPNA) as substrate using a buffer containing Tris-HCl (50 mM) and $CaCl_2$ (20 mM) at pH 8.2 (Rungruangsak-Torrissen, 1984). Production of p-nitroaniline per min was monitored at 25 °C and 410 nm. Chymotrypsin activity was determined using succinyl-Ala-Ala-Ala-Pro-Phe-p-nitroanilide (SAAPFpNA) as substrate (Rungruangsak-Torrissen, 1984). Chymotrypsin activity was measured by a similar method to the trypsin assay by quantifying the nitroaniline produced. Total protein in the intestine homogenate was quantified by the folin phenol method (Lowry et al., 1951) and used to calculate specific enzyme activities.

8. Calculations and statistical analysis

Fish performance was calculated using the tank as the experimental unit ($n = 3$). The percentage of fish survival was calculated as [(Nf/Ni) × 100], where Nf is the final number of fish and Ni is the initial number of fish. Weight gain (WG, %) was calculated as [(FBW) − (IBW) / IBW × 100], in which FBW is the average final body weight (g), and IBW is the average initial body weight (g). Daily growth index (DGI, g day) was calculated as 100 × [$(FBW)^{1/3}$ − $(IBW)^{1/3}$] × trial duration (days). Specific growth rate (SGR, %BW day^{-1}) was calculated as 100 × [ln FBW − ln IBW] / trial duration (days). Voluntary feed intake (VFI, % day^{-1}) was calculated as [FI (g) / ABW (g) / trial duration (days)], where FI (feed intake) was calculated as the total feed given during the growth trial (gDM), and ABW (average body weight) was calculated as [(IBW + FBW) / 2]. Feed conversion ratio (FCR) was calculated as [FI (gDM) / WG (g)] × 100. Protein efficiency ratio (PER) was calculated as WG (g) / crude protein intake (g). Relative growth rate on metabolic weight (RGR_m, g $kg^{0.8}$ day^{-1}) was calculated as (FBW) − (IBW) / trial duration (days) / ABW (g). Relative feeding rate on metabolic weight (RFR_m, gDM $kg^{0.8}$ day^{-1}) was calculated as FI / trial duration (days) / ABW (g). Gross energy intake (GEI, kJ $kg^{0.8}$ day^{-1}) was calculated as

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the product of absolute FI during the growth trial (gDM fish^{-1}) and gross energy content of the diet (kJ gDM^{-1}). Hepatosomatic index (HSI) was calculated as 100 × [liver weight (g) / fish weight (g)].

For analysis and calculations of metabolic parameters, the fish was used as the experimental unit ($n = 6$–12). Data was tested for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests, respectively. All the parameters were analyzed by one-way analysis of variance (ANOVA) for possible differences and comparing means using a Tukey's test ($P < 0.05$). When ANOVA assumptions were not fulfilled, a nonparametric test (Kruskal–Wallis), followed by Mann-Whitney test was used. Values are reported as means ± standard error of the mean. Data analyses were carried out using SigmaPlot 11.0 (Systat Software, USA).

9. Results

9.1. Effect of DEB on fish performance

Table 3 presents fish performance parameters calculated in meagre fed the two experimental diets for 69 days. Meagre fed the DEB 200 diet had better growth performance, as the fish exhibited a 1.8-fold increase in body weight compared to a 1.5-fold increase in the DEB 700 group. DEB 200 group displayed higher values in WG (66%), DGI (63%) and SGR (60%) comparing to DEB 700 group ($P < 0.05$). The voluntary feed intake (VFI) was different between both groups, as meagre fed the DEB 700 diet consumed approximately 30% more food than fish fed the DEB 200 diet ($P < 0.05$). A high feed-conversion ratio (FCR) estimated for meagre fed the DEB 700 diet showed that the conversion of food into body mass gain was 47.5% less efficient in DEB 700 group than in DEB 200 group ($P < 0.05$). In addition, DEB 700 group had a 52% lower protein efficiency ratio (PER) than in the DEB 200 group ($P < 0.05$). In accordance with this data, the relative feeding rate on metabolic weight (RFRm) and the gross energy intake (GEI) were 24% higher in DEB 700 group than in the DEB 200 group ($P < 0.05$). This increased efficiency in fish fed DEB 200 diet was also reflected by the 38% higher relative growth rate (RGRm) when compared to the DEB 700 group ($P < 0.05$). The HSI in meagre fed the DEB 700 diet was significantly higher than the DEB 200 group ($P < 0.05$). Fish fed DEB 200 diet showed a 3.3% ± 1.7 mortality, whereas no mortality was observed for meagre fed the DEB 700 diet.

9.2. Effect of DEB on stomach chyme characteristics

The impact of DEB levels on gastric chyme characteristics of meagre, 2 h after feeding are presented in Table 4. The gastric chyme of meagre fed the DEB 700 diet had higher dry matter content (DM) than the DEB 200 group ($P < 0.001$). Protein and energy content of the chyme was significantly higher in DEB 700 group than in the DEB 200 group ($P < 0.05$). The lipid content of the chyme was significantly lower in the DEB 700 group than in the DEB 200 group. Two hours after feeding, the gastric chyme pH in DEB 700 group was significantly higher than in DEB 200 group ($P < 0.05$).

Table 3
Effect of dietary electrolyte balance (DEB) levels on meagre performance.

Parameter	Diets		
	DEB 200	DEB 700	*P* value
Initial BW (g)	39.4 ± 0.2	39.4 ± 0.4	0.971
Final BW (g)	70.8^{a} ± 1.3	57.2^{b} ± 2.0	0.005
WG (%)	79.7^{a} ± 3.1	48.1^{b} ± 3.7	0.003
VFI (% day^{-1})	1.05^{a} ± 0.03	1.38^{b} ± 0.02	0.005
DGI	1.06^{a} ± 0.04	0.65^{b} ± 0.06	0.004
SGR (% day^{-1})	0.85^{a} ± 0.03	0.54^{b} ± 0.05	0.004
FCR	1.28^{a} ± 0.07	2.44^{b} ± 0.19	0.006
PER	1.75^{a} ± 0.09	0.91^{b} ± 0.07	0.008
RGRm (g kg$^{-0.8}$ day^{-1})	4.78^{a} ± 0.16	2.95^{b} ± 0.27	0.004
RFRm (g DM kg$^{-0.8}$ day^{-1})	6.23^{a} ± 0.22	7.70^{b} ± 0.26	0.024
GEI (kJ kg$^{-0.8}$ day^{-1})	137.4^{a} ± 4.8	169.7^{b} ± 5.8	0.024
HSI	1.91^{a} ± 0.04	2.07^{b} ± 0.01	0.025

BW, body weight; WG, Weight gain, VFI, voluntary feed intake; DGI, daily growth index; SGR, specific growth rate; FCR, feed conversion ratio; PER, protein efficiency ratio; RGRm, relative growth rate on metabolic weight; RFRm, relative feeding rate on metabolic weight; GEI, gross energy intake; DM, dry matter; HSI, hepatosomatic index. Data presented are mean ± standard error of the mean ($n = 3$). Different superscript letters in each row indicate significant differences among the experimental groups ($P < 0.05$).

Table 4
Effect of dietary electrolyte balance (DEB) levels on stomach chyme of meagre.

Parameter	Diets		
	DEB 200	DEB 700	*P* value
DM (%)	22.9^{a} ± 0.0	29.0^{b} ± 0.4	<0.001
Water content (%)	77.1 ± 2.0	71.0 ± 3.9	0.203
Total protein (% on DM)	43.6^{a} ± 0.1	45.8^{b} ± 0.7	0.011
Total Lipids (% on DM)	19.8^{a} ± 0.4	17.7^{b} ± 0.2	0.002
Energy (kJ gDM^{-1})	19.4^{a} ± 0.4	21.2^{b} ± 0.3	0.006
pH	2.9^{a} ± 0.3	3.9^{b} ± 0.3	0.023

DM, dry matter. Data presented are mean ± standard error of the mean ($n = 6$). Different superscript letters in each row indicate significant differences among the experimental groups ($P < 0.05$).

9.3. Effect of DEB on blood and plasma parameters

Post-prandial (2 h) effects of DEB diets on several blood and plasmatic parameters of meagre are presented in Table 5. Blood pH was significantly lower in the DEB 700 group than in the DEB 200 group ($P < 0.05$). Plasma Ca^{2+} and Cl^{-} concentrations were significantly higher in DEB 700 group than in DEB 200 group ($P < 0.05$). No significant differences were observed on plasma osmolality, Na^{+} or K^{+} concentration, regardless of the dietary treatment.

The post-prandial and long-term (24 h) effects of DEB diets on plasma glucose and triacylglycerol (TAG) levels in meagre are presented in Fig. 1. Fish fed DEB 700 diet showed significantly lower glucose (Fig. 1A) and TAG (Fig. 1B) concentrations than fish fed the DEB 200 diet at both sampling times ($P < 0.05$). A decrease in glucose and TAG concentrations were observed in both dietary treatments after fasting ($P < 0.05$), although the decrease was more evident on DEB 200 group.

9.4. Effect of DEB on digestive enzyme activities

As shown in Fig. 2.A, meagre fed the DEB 700 showed significantly higher intestine amylase activity than DEB 200 group ($P < 0.05$). Lipase activity (Fig. 2.B), proteases activities (trypsin and chymotrypsin, Fig. 2.C and .D, respectively) and the trypsin to chymotrypsin ratio (Fig. 2.F) were not significantly different between the dietary groups. Nevertheless, the amylase to trypsin ratio was significantly higher in meagre fed the DEB 700 than the DEB 200 group (Fig. 2.E, 2.1-fold) ($P < 0.05$).

Table 5
Effect of dietary electrolyte balance (DEB) levels on blood pH, osmolality and ion concentrations in plasma of meagre 2 h after feeding.

Parameters	Diets		
	DEB 200	DEB 700	*P* value
pH	7.3^{a} ± 0.1	6.9^{b} ± 0.1	0.009
Osmolality (mosmol)	386.6 ± 6.7	399.6 ± 3.8	0.130
Na^{+} (mM)	135.3 ± 4.1	150.4 ± 12.8	0.485
K^{+} (mM)	4.91 ± 0.57	6.75 ± 0.54	0.093
Ca^{2+} (mM)	3.55^{a} ± 0.23	5.46^{b} ± 0.46	0.004
Cl^{-} (mM)	120.2^{a} ± 4.7	138.7^{b} ± 1.6	0.004

Data presented are mean ± standard error of the mean ($n = 6$). Different superscript letters in each row indicate significant differences among the experimental groups ($P < 0.05$).

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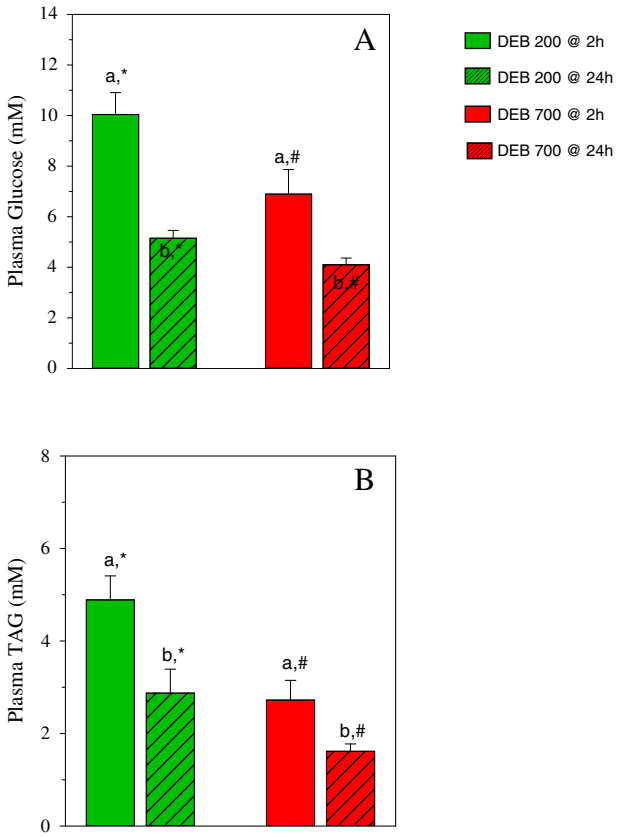

Fig. 1. Effect dietary electrolyte balance (DEB) in plasma metabolites of meagre 2 h or 24 h after feeding. (A), glucose and (B) triacylglycerol (TAG). Values are mean ± standard error of the mean ($n = 6$ for 2 h and $n = 12$ for 24 h groups). Different letters indicate significant differences among sampling time for a diet, where different symbols indicate significant differences among diets for a sampling time ($P < 0.05$).

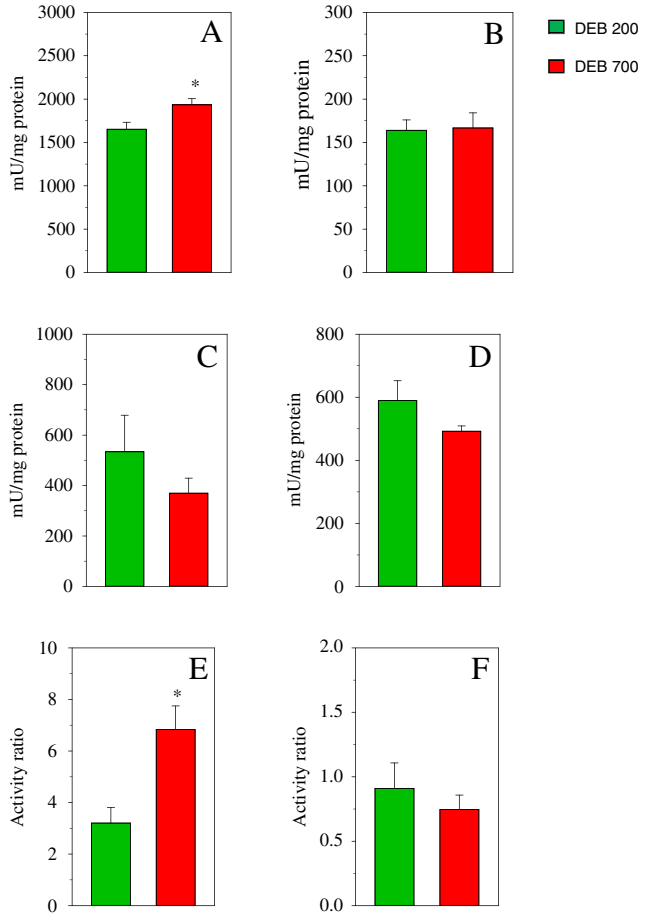

Fig. 2. Effect dietary electrolyte balance (DEB) on digestive enzyme activities in intestine of meagre 24 h after feeding. (A), amylase; (B), lipase; (C), trypsin; (D), chymotrypsin; (E), amylase to trypsin ratio; (F), trypsin to chymotrypsin ratio. Values are mean ± standard error of the mean ($n = 12$). * Significant differences between dietary treatments ($P < 0.05$).

9.5. Effect of DEB on metabolic rates

Fish fed the DEB 200 diet showed significantly higher values for routine metabolic rate (RMR) and standard metabolic rate (SMR) than fish fed DEB 700 diet ($P < 0.05$) (Table 6). No significant differences were observed for the maximal metabolic rate (MMR) or the aerobic metabolic scope (AMS) between the dietary groups.

10. Discussion

Feeding, digestion and the successive absorption of nutrients provide energy for maintenance and growth (Talbot, 1993), and any alteration to these processes may significantly alter homeostasis, consequently generating energy costs (Saravanan et al., 2012). The increase in the replacement of traditional feed ingredients in diet formulations by alternative sources has raised new questions regarding the dietary electrolyte imbalances, which has been frequently overlooked, particularly in fish nutrition. Acid-base (pH) homeostasis involves mechanisms interlinked with osmo and ion regulation that take place in gills and gastrointestinal tract (GIT) of fish. It has been suggested that osmo and ion regulation are metabolically demanding processes that affect growth and fitness of fish (Bœuf and Payan, 2001). However, the effect that dietary acid-base disruptions (e.g. induced by dietary electrolyte imbalances) have on digestion, nutrient assimilation and ultimately growth performances has barely been investigated in aquaculture fish species.

In the current study, fish fed the DEB 200 diet had 60–66% better growth performance than the DEB 700 group. However, considering that voluntary feed intake in fish fed the DEB 700 diet was 30% higher than fish fed the DEB 200 diet, the latter group was 90–96% more efficient in somatic growth than fish fed the electrolyte-imbalanced diet (DEB 700). The reduction on growth performance in meagre fed an electrolyte-imbalanced diet is consistent with a previous study in Nile tilapia (Saravanan et al., 2013). These authors found that feeding tilapia restrictively (1.4% DM of average BW) with an electrolyte-imbalanced diet (DEB 800) resulted in a 15% lower growth performance and 54% higher maintenance energy expenditure than the DEB 200 group. Nevertheless, the effects of a high DEB diet on growth performance appear to be more pronounced in meagre, most likely due to the feed consumption restriction applied for tilapia. Results from both experiments contrast with a previous study in African catfish, in which growth was positively correlated to high DEB levels (Dersjant-Li et al., 1999).

Table 6
Effect of dietary electrolyte balance (DEB) levels on metabolic rates and aerobic scope of meagre.

Parameters	Diets		
	DEB 200	DEB 700	*P* value
RMR	$157.5^{a} \pm 26.3$	$86.9^{b} \pm 3.9$	0.017
MRR	276.9 ± 26.7	245.9 ± 19.1	0.536
SMR	$99.1^{a} \pm 10.2$	$70.6^{b} \pm 4.5$	0.027
AMS	177.8 ± 24.7	175.4 ± 19.1	0.939

RMR, routine metabolic rate; MMR, maximal metabolic rate; SMR, standard metabolic rate; AMS, aerobic metabolic scope. Data presented are mean ± standard error of the mean ($n = 8$), expressed in mg O_2 kg^{-1} h^{-1}. Different superscript letters in each row indicate significant differences among the experimental groups ($P < 0.05$).

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However, these authors have also shown a mild increase of 9.4% on feed intake in catfish fed ad libitum the DEB 700 diet when compared to a DEB 100 group. Although a DEB 100 diet could be inadequate in terms of optimal DEB for catfish, differences on growth performance between the African catfish and meagre fed diets with contrasting DEB may be explained by differences in total dietary energy intake alone.

In freshwater and marine teleost, digestion alters not only acid-base balance (Bucking and Wood, 2009; Taylor and Grosell, 2009; Wood et al., 2010) but also water and ion balances (Bucking et al., 2011; Bucking and Wood, 2006, 2007; Grosell, 2010; Taylor et al., 2007; Wood and Bucking, 2010), resulting in physiological adjustments to relieve such dietary induced disruptions in homeostasis. Therefore, in the current study, differences on growth performance observed in meagre fed the different acid-base diets could be related to the higher energy requirements of fish fed alkaline diet (DEB 700) to maintain acid-base, water and ion balances. Estimates of energy use in several fish species suggest that 20% to 68% of total energy budget is allocated to osmoregulation (Bœuf and Payan, 2001). These values are lower than the difference in energy use for somatic growth between meagre fed DEB 200 and DEB 700 diets (90% less efficient in fish fed the alkaline diet). Therefore, differences in growth performance between the dietary treatments may be associated as well to a reduction in the efficiency of digestion and nutrient assimilation in fish fed the DEB 700 diet.

The digestive process starts with the gastric secretion of HCl from the parietal cells, which initiates the proteolysis required for nutrient assimilation. As a consequence of HCl gastric secretion, an increase in HCO_3^- concentration in blood occurs, producing a significant alkalization of the blood (alkaline tide) in all vertebrates, including fish (Bucking et al., 2009; Wood et al., 2005). In the gastric cells, the rate of H^+ secretion into the stomach is matched by HCO_3^- excretion into the plasma by a Cl^-/HCO_3^- exchanger. This process is catalyzed by intracellular carbonic anhydrase, which converts H_2O and CO_2 to HCO_3^- and H^+, and also provides H^+ for secretion in parallel with Cl^- secretion (Niv and Fraser, 2002). The current study showed that DEB 700 diet caused a reduction in blood pH in meagre after 2 h of feeding, indicating that DEB 700 diet (rich in HCO_3^-) decreased the intensity of the alkaline tide. The acid-base imbalance produced during digestion was linked to a higher gastric chyme pH in the group fed the alkaline (DEB 700) diet.

Dry matter, protein and energy content in the gastric chyme of meagre fed the alkaline diet were higher than in the DEB 200 group, suggesting a decrease in the efficiency of the digestive process in the DEB 700 group. This is also supported by lower glucose and TAG concentrations in plasma of meagre fed the alkaline diet at 2 and 24 h hours post-prandial, showing a noticeable metabolic effect of the dietary treatment in the post-absorptive state. We expected changes taking place in trypsin and chymotrypsin activities in the intestine and pyloric ceca of meagre fed different DEB levels, since these proteases are sensitive to changes in chyme pH in fish (Krogdahl et al., 2015). Trypsin activity is modulated by environmental and dietary conditions favoring growth (Rungruangsak Torrissen and Male, 2000). Conversely, chymotrypsin activity is modified under suboptimal conditions (e.g. starvation) when growth is limited or depressed (Rungruangsak-Torrissen et al., 2006). The trypsin to chymotrypsin ratio (T/C ratio) is a reliable marker when contrasting diet quality, digestive efficiency, and the potential growth performance in fish (Sunde et al., 2004). Surprisingly, trypsin, chymotrypsin and the T/C ratio were not altered in meagre, regardless of the dietary treatment. It can be argued that DEB may have a stronger effect on acidic protease activity, such as pepsin (Gildberg, 1988). This enzyme is the proteolytically active form of pepsinogen, which is secreted by gastric cells and autocatalytically activated in acidic environments (Bomgren et al., 1998). A previous study reveals that pepsin activity is less sensitive to changes in diet composition than trypsin and chymotrypsin in the GIT of Atlantic salmon (Rungruangsak and Utne, 1981). Future studies should elucidate the effects of DEB on protein digestibility and the subsequent absorption of amino acids and dipeptides in the GIT of meagre.

Fish fed the DEB 700 diet have a greater capacity to digest dietary carbohydrate, as indicated by the higher intestinal amylase activity and amylase/trypsin ratios than DEB 200 group. A previous study has shown that amylase activity is influenced by ion type and concentration of the fish GIT (Munilla-Moran and Saborido-Rey, 1996). Therefore, enhanced amylase activity observed in GIT of the DEB 700 group could be linked to higher pH and/or ion content of the chyme. To this end, Usher et al. (1990) showed that chyme pH in mid and hindgut of Atlantic salmon (*Salmo salar*) was higher in seawater (SW)- than in freshwater (FW)-adapted smolts. The authors proposed that the higher chyme pH, and more likely an increased ionic strength in the GIT of this species, may create differences in the apparent digestibility of both groups. Similar differences in pH and ionic strength of the chyme may be also present in meagre fed different DEB diets. However, other factors could contribute to the observed increase in amylase activity in GIT of meagre found in our study. Optimal pH for amylase activity appears to vary according to the species (Krogdahl et al., 2005) and possible isoforms present in the GIT of fish (Alarcon et al., 2001; Fernández et al., 2001; Moreau et al., 2001; Yamada et al., 1991). In addition, it is difficult to distinguish endogenous amylase secretion from other sources, such as microflora production in the GIT of fish (Krogdahl et al., 2005). Even so, the increase in intestinal amylase activity in meagre fed the DEB 700 diet may suggest that carbohydrate digestibility could be potentially increased by altering the electrolyte balance of commercial diets. Further research evaluating changes in nutrient digestibility of diets with increasing amounts of carbohydrates and DEB levels may assess this interpretation.

Plasma pH appears to be adjusted mostly by the net accumulation of HCO_3^- and secretion of acid equivalents by gill ionocytes towards the environment, which may also involve multiple mechanisms (Perry and Gilmour, 2006). According to the current models proposed, the acid-base balance in marine fish is achieved by excretion of H^+ from the gill epithelium towards the environment via an apical Na^+/H^+ exchanger and a H^+-ATPase pump. In addition, the HCO_3^- is exchanged with Cl^- and with a Na^+/HCO_3^- co-transporter, both processes taking place in the gill (Esbaugh et al., 2012; Heuer and Grosell, 2014; Hwang et al., 2011). All these mechanisms working to reestablish acid-base balance may increase the energy required for maintenance metabolism in fish. In fact, previous research focused on energy balance data showed that DEB imbalanced diets increase the maintenance energy expenditure of catfish and tilapia (Dersjant-Li et al., 1999, 2000; Saravanan et al., 2013). In accordance with these studies, the relative feeding rate on metabolic weight (RFRm) measured in meagre fed the electrolyte-imbalanced diet (DEB 700) was higher than in fish fed the balanced diet (DEB 200). Surprisingly, standard metabolic (SMR) and routine metabolic (RMR) rates were lower in fish fed the DEB 700 diet when measured 48 h after feeding. Lower pH values measured in blood of the DEB 700 group may be linked to the reduced metabolic rates (SMR and RMR), most likely by affecting the hemoglobin O_2-binding capacity (Packer, 1979; Pelster and Weber, 1991). However, respirometry measurements were implemented 48 h after feeding, which may exclude an immediate dietary effect on both SMR and RMR values. Therefore, the decrease in SMR and RMR observed in meagre fed the DEB 700 diet could be associated with a long term effect of high energetic demands imposed by acid-base regulation.

Osmolality and ion concentrations (Na^+ and K^+) in the plasma of meagre 2 h after feeding remained unchanged, regardless of the decrease of blood pH in the DEB 700 group when compared to the DEB 200 group. The concentrations of Cl^- and Ca^{2+} in plasma of fish fed the DEB 700 diet were higher than those in the DEB 200 group, which may be explained by a decreased CO_3^{2-} secretion and precipitation as CO_3Ca in the GIT, mechanisms taking place in marine fish to conserve water (Marshall and Grosell, 2005; Wilson et al., 1996, 2002). Future studies should further investigate the effect of dietary electrolyte balance on osmolality and ion content, along with the possible impact in the pH of the GIT, to elucidate the mechanisms regulating water and ion balances in meagre during the challenge.

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11. Conclusions

The current study demonstrates that feeding meagre to satiation with acidic (DEB 200) or alkaline (DEB 700) diets elicits significant changes in energy allocation for somatic growth. The ingestion of an alkaline diet produces a disruption in meagre digestive process by changing the gastric chyme pH and intestinal amylase activity, thus, affecting the digestion and the assimilation of dietary nutrients. However, lower routine metabolic (RMR) and standard metabolic (SMR) rates observed in meagre fed the DEB 700 diet cannot explain the differences in growth observed in this study. Future studies should examine the contribution of gill ionocytes towards acid-base and ion balances in fish fed different DEB diets, as well as the impact that such diets may have on apparent nutrient digestibility. These approaches would allow us to better understand how DEB impacts the energy allocated to several metabolic functions in fish.

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References

Alarcon, F.J., Martinez, T.F., Diaz, M., Moyano, F.J., 2001. Characterization of digestive carbohydrase activity in the gilthead seabream (*Sparus aurata*). Hydrobiologia 445, 199–204.

Baktoft, H., Jacobsen, L., Skov, C., Koed, A., Jepsen, N., Berg, S., Boel, M., Aarestrup, K., Svendsen, J.C., 2016. Phenotypic variation in metabolism and morphology correlating with animal swimming activity in the wild: relevance for the OCLTT (oxygen- and capacity-limitation of thermal tolerance), allocation and performance models. Conserv. Physiol. 4.

Bernfeld, P., 1951. Enzymes of starch degradation and synthesis. Advances in Enzymology and Related Subjects of Biochemistry, pp. 379–428.

Boeuf, G., 1993. Salmonid smolting: a pre-adaptation to the oceanic environment. In: Rankin, J.C., Jensen, F.B. (Eds.), Fish Ecophysiology. Chapman & Hall, London, pp. 105–135.

Bœuf, G., Payan, P., 2001. How should salinity influence fish growth? Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol. 130, 411–423.

Bomgren, P., Einarsson, S., Jönsson, A.-C., 1998. Similarities and differences in oxynticopeptic cell ultrastructure of one marine teleost, *Gadus morhua* and one freshwater teleost, *Oncorhynchus mykiss*, during basal and histamine-stimulated phases of acid secretion. Fish Physiol. Biochem. 18, 285–296.

Bucking, C., Wood, C.M., 2006. Gastrointestinal processing of Na+, Cl−, and K+ during digestion: implications for homeostatic balance in freshwater rainbow trout. Am. J. Phys. 291, R1764–R1772.

Bucking, C., Wood, C.M., 2007. Gastrointestinal transport of Ca2+ and Mg2+ during the digestion of a single meal in the freshwater rainbow trout. J. Comp. Physiol. B: Biochem. Syst. Environ. Physiol. 177, 349–360.

Bucking, C., Wood, C.W., 2009. The effect of postprandial changes in pH along the gastrointestinal tract on the distribution of ions between the solid and fluid phases of chyme in rainbow trout. Aquac. Nutr. 15, 282–296.

Bucking, C., Fitzpatrick, J.L., Nadella, S.R., Wood, C.M., 2009. Post-prandial metabolic alkalosis in the seawater-acclimated trout: the alkaline tide comes in. J. Exp. Biol. 212, 2159–2166.

Bucking, C., Fitzpatrick, J.L., Nadella, S.R., McGaw, I.J., Wood, C.M., 2011. Assimilation of water and dietary ions by the gastrointestinal tract during digestion in seawater-acclimated rainbow trout. J. Comp. Physiol. B: Biochem. Syst. Environ. Physiol. 181, 615–630.

Cárdenas, S., 2012. Biología y acuicultura de corvinas en el mundo. AquaTIC 37, 1–13.

Champagnat, C., Domain, F., 1978. Migrations des poissons demersaux le long des cotes ouest-africaines de 10° a 24° de latitude nord.

Chiu, Y.U., Austic, R.E., Rumsey, G.L., 1984. Effect of dietary electrolytes and histidine on histidine metabolism and acid-base balance in rainbow trout (Salmo Gairdneri). Comp. Biochem. Physiol. B 78, 777–783.

Chiu, Y.N., Austic, R.E., Rumsey, G.L., 1988. Effect of feeding level and dietary electrolytes on the arginine requirement of rainbow trout (Salmo Gairdneri). Aquaculture 69, 79–91.

Claiborne, J.B., Edwards, S.L., Morrison Shetlar, A.I., 2002. Acid-base regulation in fishes: cellular and molecular mechanisms. J. Exp. Zool. 293, 302–319.

Cooper, C.A., Wilson, R.W., 2008. Post-prandial alkaline tide in freshwater rainbow trout: effects of meal anticipation on recovery from acid-base and ion regulatory disturbances. J. Exp. Biol. 211, 2542–2550.

Dersjant-Li, Y., Verreth, J.A.J., Evers, F., Tijssen, P.A.T., Booms, R., Verstegen, M.W.A., 1999. The influence of dietary cation-anion differences on acid-base balance, food intake, growth and nutrient utilisation of juvenile African catfish *Clarias gariepinus* (Burchell). Fish Physiol. Biochem. 20, 305–311.

Dersjant-Li, Y., Verreth, J.A.J., Tijssen, P.A.T., Booms, R., Verstegen, M.W.A., Huisman, E.A., 2000. Metabolic costs of changing the cation-anion difference in the diet of juvenile African catfish *Clarias gariepinus* (Burchell). Aquac. Nutr. 6, 39–45.

Dersjant-Li, Y., Verreth, J.A.J., Schrama, J.W., Huisman, E.A., Verstegen, M.W.A., 2001. The impact of the dietary electrolyte balance on growth performance, energy metabolism and acid-base balance: a comparison between African catfish (*Clarias gariepinus*) and pigs. Aquac. Res. 198, 293–305.

Duncan, N., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C., Roo, F., Schuchardt, D., Vallés, R., 2013. Aquaculture production of meagre (*Argyrosomus regius*): hatchery techniques, ongrowing and market. In: Allan, G., Burnell, G. (Eds.), Advances in Aquaculture Hatchery Technology. Woodhead, Cambridge, pp. 519–541.

Esbaugh, A.J., Heuer, R., Grosell, M., 2012. Impacts of ocean acidification on respiratory gas exchange and acid-base balance in a marine teleost, *Opsanus beta*. J. Comp. Physiol. B. 182, 921–934.

Evans, D.H., Permarini, P.M., Choe, K.P., 2005. The multifunction of fish gill: dominant site of gas exchange, osmoregulation, acid-base regulation, and excretion of nitrogenous waste. Physiol. Rev. 85, 97–177.

Fernández, I., Moyano, F.J., Díaz, M., Martínez, T., 2001. Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). J. Exp. Mar. Biol. Ecol. 262, 1–12.

Genz, J., Taylor, J.R., Grosell, M., 2008. Effects of salinity on intestinal bicarbonate secretion and compensatory regulation of acid–base balance in *Opsanus beta*. J. Exp. Biol. 211, 2327–2335.

Genz, J., Jyde, M.B., Svendsen, J.C., Steffensen, J.F., Ramløv, H., 2013. Excess post-hypoxic oxygen consumption is independent from lactate accumulation in two cyprinid fishes. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 165, 54–60.

Gildberg, A., 1988. Aspartic proteinases in fishes and aquatic invertebrates. Comp. Biochem. Physiol. Part B: Comp. Biochem. 91, 425–435.

Gilmour, K.M., Perry, S.F., Esbaugh, A.J., Genz, J., Taylor, J.R., Grosell, M., 2012. Compensatory regulation of acid–base balance during salinity transfer in rainbow trout (*Oncorhynchus mykiss*). J. Comp. Physiol. B. 182, 259–274.

González-Quirós, R., del Árbol, J., García-Pacheco, M., Silva-García, A., Naranjo, J., Morales-Nin, B., 2011. Life-history of the meagre *Argyrosomus regius* in the Gulf of Cádiz (SW Iberian Peninsula). Fish. Res. 109, 140–149.

Grosell, M., 2010. The role of the gastrointestinal tract in salt and water balance. Fish Physiol. 135–164.

Heuer, R.M., Grosell, M., 2014. Physiological impacts of elevated carbon dioxide and ocean acidification on fish. Am. J. Physiol. Regul. Integr. Comp. Physiol. 307, R1061–R1084.

Hwang, P.-P., Lee, T.-H., Lin, L.-Y., 2011. Ion regulation in fish gills: recent progress in the cellular and molecular mechanisms. Am. J. Physiol. Regul. Integr. Comp. Physiol. 301, R28–R47.

Janssen, R.G., Randall, D.J., 1975. The effects of changes in pH and PCO_2 in blood and water on breathing in rainbow trout, Salmo Gairdneri. Respir. Physiol. 25, 235–245.

Krogdahl, Å., Hemre, G.I., Mommsen, T.P., 2005. Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. Aquac. Nutr. 11, 103–122.

Krogdahl, Å., Sundby, A., Holm, H., 2015. Characteristics of digestive processes in Atlantic salmon (*Salmo salar*). Enzyme pH optima, chyme pH, and enzyme activities. Aquaculture 449, 27–36.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurementwith the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Marshall, W.S., Grosell, M., 2005. Ion transport, osmoregulation and acid-base balance. In: Evans, D., Claiborne, J.B. (Eds.), Physiology of Fishes. CRC Press, Boca Raton, pp. 177–230.

Moreau, Y., Desseaux, V., Koukiekolo, R., Marchis-Mouren, G., Santimone, M., 2001. Starch digestion in tropical fishes: isolation, structural studies and inhibition kinetics of a-amylases from two tilapias *Oreochromis niloticus* and *Sarotherodon melanotheron*. Comp. Biochem. Physiol. B 128, 543–552.

Moutou, K.A., Panagiotaki, P., Mamuris, Z., 2004. Effects of salinity on digestive protease activity in the euryhaline sparid *Sparus aurata* L.: a preliminary study. Aquac. Res. 35, 912–914.

Munilla-Moran, R., Saborido-Rey, F., 1996. Digestive enzymes in marine species. II. Amylase activities in gut from seabream (*Sparus aurata*), turbot (*Scophthalmus maximus*) and redfish (*Sebastes mentella*). Comp. Biochem. Physiol. B 113, 827–834.

Nikolopoulou, D., Moutou, K.A., Fountoulaki, E., Venou, B., Adamidou, S., Alexis, M.N., 2011. Patterns of gastric evacuation, digesta characteristics and pH changes along the gastrointestinal tract of gilthead sea bream (*Sparus aurata* L.) and European sea bass (*Dicentrarchus labrax* L.). Comp. Biochem. Physiol. A Mol. Integr. Physiol. 158, 406–414.

Niv, Y., Fraser, G.M., 2002. Esophageal and gastric diseases; the alkaline tide phenomenon. J. Clin. Gastroenterol. 35, 5–8.

Packer, R.K., 1979. Acid-base balance and gas exchange in brook trout (*Salvelinus fontinalis*) exposed to acidic environments. J. Exp. Biol. 79, 127–134.

Patience, J.F., Austic, R.E., Boyd, R.D., 1987. Effect of dietary electrolyte balance on growth and acid-base status in swine. J. Anim. Sci. 64, 457–466.

Peixoto, M.J., Svendsen, J.C., Malte, H., Pereira, L.F., Carvalho, P., Pereira, R., Gonçalves, J.F.M., Ozório, R.O.A., 2016. Diets supplemented with seaweed affect metabolic rate, innate immune, and antioxidant responses, but not individual growth rate in European seabass (*Dicentrarchus labrax*). J. Appl. Phycol. 28, 2061–2071.

Pelster, B., Weber, R.E., 1991. The physiology of the root effect. Advances in Comparative and Environmental Physiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 51–77.

Perez, K., Munch, S., 2015. Sustained costs of growth and the trajectory of recovery. Funct. Ecol. 29, 393–403.

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Perry, S.F., Gilmour, K.M., 2006. Acid-base balance and CO_2 excretion in fish: unanswered questions and emerging models. Respir. Physiol. Neurobiol. 154, 199–215.
Poli, B.M., Parisi, G., Zampacavallo, G., Iurzan, F., Mecatti, M., Lupi, P., Bonelli, A., 2003. Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits and freshness changes in refrigerated commercial-size fish. Aquac. Int. 11, 301–311.
Psochiou, E., Mamuris, Z., Panagiotaki, P., Kouretas, D., Moutou, K.A., 2007. The response of digestive proteases to abrupt salinity decrease in the euryhaline sparid *Sparus aurata* L. Comp. Biochem. Physiol. B: Biochem. Mol. Biol. 147, 156–163.
Quéméner, L., Suquet, M., Mero, D., Gaignon, J.L., 2002. Selection method of new candidates for finfish aquaculture: the case of the French Atlantic, the channel and the North Sea coasts. Aquat. Living Resour. 15, 293–302.
Quero, J.C., Vayne, J.J., 1987. Le maigre, *Argyrosomus regius* (Asso, 1801) (Pisces, Perciformes, Sciaenidae) du golfe de Gascogne et des eaux plus septentrionales. Rev. Trav. Inst. Pêches Marit. 19, 35–66.
Rosewarne, P.J., Wilson, J.M., Svendsen, J.C., 2016. Measuring maximum and standard metabolic rates using intermittent-flow respirometry: a student laboratory investigation of aerobic metabolic scope and environmental hypoxia in aquatic breathers. J. Fish Biol. 88, 265–283.
Rungruangsak Torrissen, K., Male, R., 2000. Trypsin isozymes: development, digestion and structure. In: Haard, N.F., Simpson, B.K. (Eds.), Seafood Enzymes, Utilization and Influence on Postharvest Seafood Quality. Marcel Dekker Inc., New York, pp. 215–269.
Rungruangsak, K., Utne, F., 1981. Effect of different acidified wet feeds on protease activities in the digestive tract and on growth rate of rainbow trout (Salmo Gairdneri Richardson). Aquaculture 22, 67–79.
Rungruangsak-Torrissen, K., 1984. Characterization of proteases in digestive-tract of Atlantic salmon (*Salmo salar*) in comparison with rainbow trout (Salmo Gairdneri). Comp. Biochem. Physiol. B 77, 669–674.
Rungruangsak-Torrissen, K., 2007. Digestive efficiency, growth and qualities of muscle and oocyte in Atlantic salmon (*Salmo salar* L.) fed diets with krill meal as an alternative protein source. J. Food Biochem. 31, 509–540.
Rungruangsak-Torrissen, K., Moss, R., Andresen, L.H., Berg, A., Waagbø, R., 2006. Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 32, 7–23.
Saravanan, S., Schrama, J.W., Figueiredo-Silva, A.C., Kaushik, S.J., Verreth, J.A.J., Geurden, I., 2012. Constraints on energy intake in fish: the link between diet composition, energy metabolism, and energy intake in rainbow trout. PLoS One 7, e34743.
Saravanan, S., Geurden, I., Orozco, Z.G.A., Kaushik, S.J., Verreth, J.A.J., Schrama, J.W., 2013. Dietary electrolyte balance affects the nutrient digestibility and maintenance energy expenditure of Nile tilapia. Br. J. Nutr. 110, 1948–1957.
Sunde, J., Eiane, S.A., Rustad, A., Jensen, H.B., Opstvedt, J., Nygard, E., Venturini, G., Rungruangsak-Torrissen, K., 2004. Effect of fish feed processing conditions on digestive protease activities, free amino acid pools, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.). Aquac. Nutr. 10, 261–277.
Tacon, A.G.J., De Silva, S.S., 1983. Mineral composition of some commercial fish feeds available in Europe. Aquaculture 31, 11–20.
Talbot, C., 1993. Some aspects of the biology of feeding and growth in fish. Proc. Nutr. Soc. 52, 403–416.
Taylor, J.R., Grosell, M., 2006. Feeding and osmoregulation: dual function of the marine teleost intestine. J. Exp. Biol. 209, 2939–2951.
Taylor, J.R., Grosell, M., 2009. The intestinal response to feeding in seawater gulf toadfish, *Opsanus beta*, includes elevated base secretion and increased epithelial oxygen consumption. J. Exp. Biol. 212, 3873–3881.
Taylor, J.R., Whittamore, J.M., Wilson, R.W., Grosell, M., 2007. Postprandial acid-base balance and ion regulation in freshwater and seawater-acclimated European flounder, *Platichthys flesus*. J. Comp. Physiol. B: Biochem. Syst. Environ. Physiol. 177, 597–608.
Tucker, J.J., 1998. Marine Fish Culture. Kluwer Academic Press, Norwell.
Usher, M.L., Talbot, C., Eddy, F.B., 1990. Effects of transfer to seawater on digestion and gut function in Atlantic salmon smolts (*Salmo salar* L.). Aquaculture 90, 85–96.
Wilson, R.W., Gilmour, K.M., Henry, R.P., Wood, C.M., 1996. Intestinal base excretion in the seawater-adapted rainbow trout: a role in acid-base balance? J. Exp. Biol. 199, 2331–2343.
Wilson, R.W., Wood, C.M., Gonzalez, R.J., Patrick, M.L., Bergman, H.L., Narahara, A., Val, A.L., 1999. Ion and acid-base balance in three species of Amazonian fish during gradual acidification of extremely soft water. Physiol. Biochem. Zool. 72, 277–285.
Wilson, R.W., Wilson, J.M., Grosell, M., 2002. Intestinal bicarbonate secretion by marine teleost fish - why and how? Biochim. Biophys. Acta 1566, 182–193.
Wilson, J., Reis-Santos, P., Fonseca, A.-V., Antunes, J., Bouça, P., Coimbra, J., 2007. Seasonal changes in ionoregulatory variables of the glass eel *Anguilla anguilla* following estuarine entry: comparison with resident elvers. J. Fish Biol. 70, 1239–1253.
Winkler, U.K., Stuckmann, M., 1979. Glycogen, hyaluronate, and some other polysaccharides greatly enhance the formation of exolipase by *Serratia marcescens*. J. Bacteriol. 138, 663–670.
Wood, C.M., 1991. Acid-base and ion balance, metabolism, and their interactions, after exhaustive exercise in fish. J. Exp. Biol. 160, 285–308.
Wood, C.M., Bucking, C., 2010. The role of feeding in salt and water balance. Fish Physiol. 165–212.
Wood, C.M., Marshall, W.S., 1994. Ion balance, acid-base regulation, and chloride cell function in the common killifish, *Fundulus heteroclitus*—a euryhaline estuarine teleost. Estuaries 17, 34–52.
Wood, C.M., Turner, J.D., Graham, M.S., 1983. Why do fish die after severe exercise? J. Fish Biol. 22, 189–201.
Wood, C.M., Kajimura, M., Mommsen, T.P., Walsh, P.J., 2005. Alkaline tide and nitrogen conservation after feeding in an elasmobranch (*Squalus acanthias*). J. Exp. Biol. 208, 2693–2705.
Wood, C.M., Bucking, C., Grosell, M., 2010. Acid-base responses to feeding and intestinal Cl- uptake in freshwater- and seawater-acclimated killifish, *Fundulus heteroclitus*, an agastric euryhaline teleost. J. Exp. Biol. 213, 2681–2692.
Yamada, A., Takano, K., Kamoi, I., 1991. Purification and properties of amylases from tilapia intestine. Nippon Suisan Gakkaishi 57, 1903–1909.

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