Gian Marco Dardengo

How to improve fish robustness through nutritional supplementation in fish larvae

Academic year 2019/2020

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MSc. Aquaculture and Fisheries

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Declaro ser o autor deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam na listagem de referências incluída.

Universidade do Algarve, 30 de setembro de 2020

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"Heroes come and go, but legends are forever."

#Mambamentality, #Mambaforever

ABSTRACT

The concept of nutritional programming began to arouse interest around larviculture sector due to the high metabolic plasticity of larvae. At this life stage, specific methabolic pathways of young organisms are more proned to be alterated with effects that may be propagated in the long terms. The aim of the present study was to assess the effect of dietary curcumin as promoter of both gut maturation and antioxidant status of gilthead seabream larvae (*Sparus aurata*). Curcumin was delivered through microdiet since mouth opening (4 Days After Hatching, DAH) in two levels of supplementation (LOW and HIGH) and the effects were compared with larvae fed on commercial diet (CTRL). Feeding plan consisted of a short period of co-feeding with live preys (rotifers and *Artemia* nauplii) and inert diet until 24 DAH, when larvae were weaned. Results on survival rate showed that, curcumin did not influence this paramenter. Key performance parameters did not reveal statistical differences between treatments, although a positive trend was detected in larvae fed on LOW curcumin supplementation. Proteolytic enzymes, such as trypsin and chymotrypsin, were positively influenced by curcumin being significantly higher in larvae fed on HIGH curcumin supplementation. Despite this, curcumin did not influence the activity of the remain digestive enzymes analysed (aminopeptidase, amylase, 4C and 18C-like lipases and alkaline phosphatase). Curcumin did not change the larval feeding habits or diet palatability; results revealed that weaning larvae at 24 DAH did not influence their feeding incidence. Overall, antioxidant status biomarkers (TG, TAC, PC and MOS) did not reveal significant differences between treatments. In summary, although results did not prove significant effects of curcumin on most of the parameters under investigation, some positive trends leave open the possibility of further investigations. These future trials may be addressed independently, or in combination, on both, early programming and new dietary additives, to test different curcumin concentrations.

Keywords: Antioxidant status, Growth, Metabolic programming, Nutrition, Plant extracts.

RESUMO

O conceito de programação nutricional refere-se a possíveis estímulos durante fases precoces do desenvolvimento do animal que irão ter repercussões em fases mais tardias da vida do mesmo. O conhecimento dos mecanismos que controlam o desenvolvimento e o crescimento e sua relação com a nutrição são fundamentais para a identificação de fases de desenvolvimento que introduzam variação de crescimento, que impactem o potencial do mesmo e/ou que afetem a viabilidade e a qualidade dos juvenis. A perspectiva de aplicar este conceito à aquacultura oferece inúmeras possibilidades, principalmente focadas na modulação de vias metabólicas, tais como a acreção proteica, a homeostase oxidativa e a maturação precoce do sistema digestivo. A nutrição é o factor ambiental mais importante que determina o crescimento e o desenvolvimento dos animais. Nos últimos anos, foi reportado que a inclusão de extractos de origem vegetal em alimentos inertes estimula o apetite e promove o ganho de peso em peixes devido a moléculas bioactivas. O objetivo do presente estudo foi avaliar o efeito da inclusão de curcumina na dieta para larvas de dourada (*Sparus aurata*) como promotora da maturação intestinal e do estado redox, e melhorar o conhecimento das várias vias fisiológicas que medeiam as relações entre dieta, nutrição e metabolismo, apontando para a biologia oxidativa, a capacidade digestiva e a plasticidade do crescimento. A curcumina foi suplementada nas microdietas desde abertura da boca (4 dias após eclosão, DAE) em dois níveis de suplementação (LOW e HIGH), os efeitos foram comparados com larvas alimentadas com dieta comercial (CTRL). O plano alimentar consistiu em um curto período de co-alimentação com presas vivas (rotíferos e *Artemia* nauplii) e dieta inerte até os 24 DAE. Após esta idade as larvas foram alimentadas exclusivamente com dieta inerte. A taxa de sobrevivência confirmou que a curcumina não afetou este parâmetro. Os principais indicadores de desempenho de crescimento não revelaram diferenças estatísticas entre os tratamentos, embora tenha sido observado uma tendência positiva em larvas alimentadas com suplementação de curcumina LOW. As enzimas proteolíticas, como a tripsina e a quimiotripsina, foram positivamente influenciadas pela curcumina sendo a actividade enzimática significativamente mais elevada em larvas alimentadas com suplementação HIGH de curcumina. A suplementação de curcumina não influenciou a actividade das restantes enzimas digestivas analisadas (aminopeptidasa, amilasa, lipasas e fosfatasa alcalina). A curcumina não alterou os hábitos alimentares das larvas ou a palatabilidade da dieta; os resultados revelaram alteração na sua incidência alimentar quando alimentadas exclusivamente com dieta inerte. No geral, os biomarcadores do estado redox (TG, TAC, PC e MOS) não revelaram diferenças significativas entre os tratamentos. Em resumo, embora os resultados não tenham demonstrado efeitos significativos da curcumina na maioria dos parâmetros sob investigação, algumas tendências positivas deixam em aberto a possibilidade de novas investigações. Esses ensaios futuros podem ser abordados de forma independente ou combinada, tanto na programação inicial quanto em novos aditivos alimentares, para testar diferentes concentrações de curcumina. Os resultados serão traduzidos em estratégias alimentares eficazes de modo a promover a robustez e a resiliência dos peixes num futuro próximo e a transferir e aplicar o conhecimento e a tecnologia de modo a garantir o desenvolvimento de um sector mais sustentável, vital para o futuro da indústria da aquacultura.

Palavras-chave: Crescimento, Estado antioxidante, Extractos de plantas, Nutrição, Programação metabólica.

Abbreviations

am - *ante meridiem*
ANOVA - analysis of variance
CCK – cholecystokinin
cm - centimeters
CTRL - control
DAH – days after hatching
DNA - Deoxyribonucleic acid
DW – dry weight
EU – European Union
g – grams
GR – glutathione reductase
GSH - reduced glutathione
IAP – intestinal alkaline phosphatase
IGF - Insulin-like growth factor
K – condition factor
kg – kilograms
L – liters
LAS – Leica Application Suite
ln - natural logarithm
m – meters
M - molar
mg – milligrams
min - minutes
mL - milliliters
mm – millimeters
mM – millimolar
mmol - millimol
MOS – mitochondrial oxidative status
n – number
nm – nanometers
P - p-value
PC – protein carbonylation
pm - *post meridiem*
PMS - post-mitochondrial supernatant
PTMs - posttranslational modifications
RFU – relative fluorescence units
RGR – relative growth rate
RNA - Ribonucleic acid
ROS – reactive oxygen species
SD – standard deviation
TAC – total antioxidant capacity
TG – total glutathione
TL – total length
T:C - trypsin:chymotrypsin ratio
USD - United States of America dollars
UV – ultraviolet
µg - micrograms
µL - microliter
µm- micrometer
µM - micromolar

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1. Introduction

1.1 World and Mediterranean aquaculture

Fisheries and aquaculture are largely recognized to be crucial activities as supplier of healthy food, employment, recreation activities, trade and economic well being for thousand of people around the world (FAO, 2020a). In fact, when compared to terrestrial animal sources of food, seafood production have received an increasing attention as source of protein and micronutrients, due to it potentially lower environmental impact (Hicks et al., 2019; Parker et al., 2018; Poore and Nemecek, 2018). As consequence, seafood might achieve food security and improved nutrition and empower both international trade and economy of the richer countries as well as the local economies of the low-income countries (Asche et al., 2015; Belton et al., 2018; Béné et al., 2015; Beveridge et al., 2013; High Level Panel of Experts on World Food Security, 2014; Röös et al., 2017). Finally, due to the trans-national and trans-generational effects of these activities, concerning not only the whole international community but affecting also both present and future generations, they should be conducted in responsible and sustainable manner (FAO, 2020a).

Aquaculture, i.e. *the farming of aquatic organisms including fish, molluscs, crustaceans and aquatic plants*, is among the fastest growing animal industries (Grigorakis and Rigos, 2011). In fact, if in the early 50's of the last century, world aquaculture production measured as millions of tonnes of live weight (plants and algae excluded) counted for just one unit, it passes from 55.1 million to 80.0 million between the years 2009 and 2016 (FAO, 2019). In 2018, last data available indicated that world aquaculture production reached the value of 82.1 million of tonnes (FAO, 2020b). Aquaculture production is still today characterized by an evident variance between countries; in fact fish farming is predominant in Asia where, over the last 20 years, has been concentrated the 89 percent of the global world production (FAO, 2020b). The contribution of aquaculture to global fish production was 46 percent in 2018 (FAO, 2020b) with the prevision to rise until 62 percent for the year 2030; accordingly to this expectation, innovation and technological improvement within the sector, as well as social acceptance, must be found and applied (FAO, 2020a). One way to reach a higher level of environmental and economical sustainability for the aquaculture sector is to reduce the Fish in : Fish out (FIFO) ratio (Kok et al., 2020; Mastoraki et al., 2020; Wang et al., 2020). In fact, with the introduction of new feed ingredients and the conceptualization of new feed formulation, it will be possible substitute, or at least reduce, fish meal and fish oil content, cutting down at the end their negative impacts (Barazi Yeroulanos, 2010; Basto et al., 2020; Belghit et al.,

2019; Campos et al., 2020; Hua and Bureau, 2012; Machado et al., 2020; Rocha et al., 2016; Teodósio et al., 2020; Yadav et al., 2020).

European aquaculture production started in the late 80's of the last century, followed by a rapid growth during the 90's (Llorente et al., 2020). Within the Mediterranean area, the larger producers, in terms of volume per year, are Egypt, Spain, Turkey France, Italy and Greece (FAO, 2020b; OECD, 2020). The most relevant farmed marine fish species are gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) produced in countries around the Mediterranean basis, and Atlantic salmon (*Salmo salar*), for which the production is mainly in Norway (Moretti et al., 2005; Rad, 2007; STECF, 2018). Focusing on European seabass and gilthead seabream industry, 95 percent of its production takes place within the Mediterranean region, being both these species part of the local *cuisine*-culture. Neverthelles, some countries like Italy, Spain, France and Portugal are not able to satisfy the national demand that is match by imports coming from Turkey, Egypt and Greece. In 2016, Europeas seabass production was 82 thousand tonnes valued at 555 million USD, while gilthead seabream production was of 82 thousand tonnes valued at 493 million of USD. Turkey is the main producer of both these species at global level (FAO, 2018), playing a key role since the beginning of the new millennium thanks to the custom agreement signed with UE on March 6, 1995. This has permitted to Turkish items to be freely traded within the EU countries, helping to expand the internal demand and to lead to the drops of the domestic market price. Indeed not only Turkey, but also Greece and others non-EU countries, took profit from their comparative advantages and are competing nowadays with many EU companies (Arikan and Aral, 2019; Kok et al., 2020; Rad, 2007; Rad and Köksal, 2000; Regnier and Bayramoglu, 2017; STECF, 2018; STEFC, 2015). Nonetheless, within the EU countries, aquaculture is recognized to have a key role in the economic activity, able not only to produce sustainable seafood but also to create employment in costal and rural areas (Guillen et al., 2015). Because of this, aquaculture was inserted into the EU's Blue Growth Strategy with noticeable public and private investment, leading to positive development in production, processing, logistic and marketing. These actions are expected to help industry profitability through demand generation and cost-saving during the future years (GLOBEFISH, 2017); in this prospect, encouraging signals appeared during the years 2015 and 2016 where the quantities produced by EU members increased significantly (Llorente et al., 2020).

1.1.2 Gilthead seabream in Mediterranean aquaculture

Gilthead seabream (*Sparus aurata*) (from Latin, *sparus* = a fish, and *aurata* = golden, 'fish with golden head') ('dourada' in Portuguese) is a marine or brackish and demersal fish, distributed in the Eastern Atlantic and Mediterranean area (Bauchot, 1990), also reported in Black sea (Magoulas et al., 1995). It is common to find gilthead seabream in sea-grass beds as well as in rocky and sandy bottom at different depth, usually ranging 1-30 m for the individuals that live in aggregated form (until 150 m for some solitary adults). It is mainly carnivorous, feeding on shellfish like mussels and oyster but also on small fish and crustaceans.

Traditionally, gilthead seabream aquaculture systems were based in coastal lagoons and brackish ponds in southern Europe while after the '80s sea cage farming was implemented and largely applied. Today, gilthead seabream is produced mainly in intensive systems based both on land and in floating cages (Jobling, 2011; Trujillo et al., 2012). These facilities are constructed to maximize growth performance and health status. Seabream reach 400g after 2 year while its commercial size ranges from 250g to more than 1500g (Føre et al., 2018). The production cycle process begins with larval rearing, which is performed in modern and highly sophisticated hatcheries. The larvae absorb the yolk sac in a few days (generally six days) and exogenous feeding begins, based initially on rotifers and *Artemia*. At 50/55 days after hatching (DAH), inert feed of 300-500 μm is introduced in the feeding plan, following the normal "late-weaning" protocol utilized by Hellenic marine fish hatcheries (Pantazis et al., 2014). The all on-growing phase is based on the supply of commercial pellets with high level of protein and energy content. Spain and Italy are the main markets of Mediterranean area in terms of pro-capita consumption (FEAP, 2014).

1.2 Marine fish larvae nutrition and early programming

The first feeding stages of aquatic organisms are extremely important for the overall success of the rearing process and, for most of the cultured aquatic species, the production of high quality larvae and juveniles is one of the main bottlenecks (Vadstein et al., 2013). In fact, some of the problems associated with larvae and juvenile quality become visible only during the following stages, where the final products can be definitely compromised (Logue et al., 2000). The main problems affecting both larvae and juveniles are correlated with poor growth performance, survival and appearance of malformation (Kolkovski et al., 2009; Vadstein et al., 2007). Different factors have been pointed out as causes of such conditions, including poor gametes quality, inadequate nutrition, suboptimal physiochemical conditions and detrimental fish-microbe interactions (Vadstein et al., 2013).

For what concern the nutritional aspect, the production of marine fish larvae and juveniles in commercial hatcheries is still based on the supply of live preys, such as rotifers and *Artemia* (Jobling, 2016). The running and the maintenance of larviculture sector represent a cost, both in terms of economy and time, and it requires technologies and trained employers (Lee, 2003; Massa, 2017). Among the solutions, in order to reduce the aquaculture production costs, there is the chance of anticipating the weaning time, i.e. *the introduction of inert diet in substitution for live prey* (Fezzardi et al., 2013). If the mentioned substitution does not represent a big challenge for freshwater fish larvae, since they can be fed on inert diet as early as mouth opening, the same can not be applied to marine fish larvae, where weaning is still performed several days after the beginning of exogenous feeding, and before that time the nutrition is based on live preys (Barazi Yeroulanos, 2010).

Among the main constraints that interfere the possibility of anticipating the weaning time, the maturation of digestive system, and consequently the digestive capacity, plays a central role. In fact, during larvae development, digestive system is not completed yet, so digestion capacity is limited. This happen in Senegalese sole (*Solea senegalensis*) larvae for example, where the proteolytic capacity was a limiting factor for protein digestion especially during the pelagic phase limitating the adaptation of larvae to inert diet (Engrola et al., 2010, 2009). To overcome this limitation different strategies are utilized, such as the manipulation of protein, both in terms of quality and complexity (Canada et al., 2019), or throught the application of nutritional programming concept (Xu et al., 2019; Zambonino-Infante et al., 2019). Early nutritional programming studies investigate novel feed additives that might be able to stimulate the development of the digestive system and enzymatic secretion. Trials performed on different species showed that a dietary stimulus applied during a critical developmental stage early in life (neonatal or post-natal nutrition) might have long-term consequences on physiological functions in later life (Burdge and Lillycrop, 2010; Daprà et al., 2011; Geurden et al., 2009; Metges et al., 2014; Patel and Srinivasan, 2002; Vagner et al., 2019). As examples: yellow perch (*Perca flavences*) juveniles performed higher weight gain if fed on a soybean based diet as first feeding (Kemski et al., 2018), or vegetable oil supplementation on seabream broodstocks diet was able to improve the progeny ability to utilize low fish meal and fish oil diets (Izquierdo et al., 2015) or more, Atlantic salmon (*Salmo salar*) fed for three weeks on plant based diet as first exogenous feeding reached higher growth rate and feed efficiency in fifteen weeks-fish challenged with 0% fish oil content diet for six weeks (Clarkson et al., 2017).

The “imprinting” of the nutritional programming events occurs through epigenetic modifications. For epigenetic is intended, i.e. *the study of mitotically heritable yet potentially*

reversible, molecular modification to DNA and chromatin without alteration to the underlying DNA sequence (Anderson et al., 2012; Li, 2002; Reik et al., 2001). These modifications can occur throughout the life cycle, in particular during the early life stages of development, and are heavily influenced by external factors (Matzke and Birchler, 2005; Reik et al., 2001). For example, some nutritional cues are labeled to be responsible of DNA methylation, which is one of the most widely studied form of epigenetic modification (Anderson et al., 2012; Zhang, 2015). The main objective to induce such modifications is to generate long-term alteration at molecular, and or metabolic level, able to give further positive results, such as improved growth performance (Fang et al., 2014; Gong et al., 2015; Rocha et al., 2015). Despite this, the knowledge about long-lasting effects of a nutritional event applied during the earliest stage of larvae development is still limited (Rocha et al., 2016).

1.3 The onset of exogenous feeding and larval digestive system

The ontogeny of the digestive system has been largely studied in many marine teleosts; confirming that the organs differentiation patterns are very similar between species, while small variations are mainly correlated with the temporal sequence of appearance of the different structures. In fact, at hatching time most of marine fish larvae have a very rudimentary digestive system without mouth and unpigmented eyes. At this stage, the digestive tract is anatomically a simple undifferentiated straight tube with an epithelium formed by a monostratified layer of columnar or cubical cells (Zambonino Infante and Cahu, 2001). The onset of exogenous feeding, i.e. *the passage from endogenous feeding* (based on nutrients contained in the yolk-sac) *to exogenous feeding*, after the complete absorption of the yolk sac and mouth opening, is a crucial period in terms of larvae survival rate and future growth performance. In fact, at this moment different variables, mainly related to nutrition and feeding aspects, such as inappropriate feed quality and feeding protocols, but also rearing conditions, may have negative impacts on the survival rate reducing, at the end, the number and quality of fish. In addition, any limitation in terms of energy uptake during feeding onset period not only affects the correct development of larvae but also compromises the growth and survival rates of the following stages of life (Valente et al., 2013). The main reasons for which this happens are linked not only to the partial development of the digestive tract, and the consequent incomplete settling of enzymatic activity at hatching time, but also with all the anatomical feature that are required to start the exogenous feeding, such as the ones related with prey localization (eyes and chemosensory organs) and capture (mouth and swimming capacity), not fully developed at that time (Cara et al., 2003; Navarro-Guillén et al., 2015),

Since mouth opening, and after live preys ingestion, organs related with ingestion-digestion activities show an allometric increase (Yúfera and Darias, 2007). Subsequently, these organs are

characterized by a fast growth and differentiation necessary to reinforce digestion and nutrient absorption during the following days (Osse et al., 1997; Sala et al., 2005). At this phase, although the digestive tract is not completely developed, the differenciated organs are entirely functional at the moment of first exogenous feeding where the digestion occurs in an alkaline environment (Yúfera and Darias, 2007). In fact the absorption of lipid and protein, occuring respectively in midgut and in hidgut enterocytes, can be observed several hours after start of feeding (Diaz et al., 2002). The glands annexed to digestive system, such as liver, pancreas and gall bladder, are also functional at mouth opening (Chen et al., 2006); as demonstration, the activity of pancreatic enzymes (trypsin, lipases and amylase) has been biochemically detected at first feeding and in many marine fish even before mouth opening (Chen et al., 2006; Elbal et al., 2004; Gisbert et al., 2004; Navarro-Guillén et al., 2015). Cytosolic enzymes of the enterocyte (amino peptidase, acid and alkaline phosphatases, esterases) are also present at first feeding in larvae of both Japanese flounder (*Paralichthys olivaceus*) and Yellowtail amberjack (*Seriola lalandi)* (Bolasina et al., 2006; Chen et al., 2006). Digestive regulatory peptides, such as acid and alkaline proteases, leucine-aminopeptidase, acid and alkaline phospatase, and hormones, such as cholecystokinin (CCK), have also been detected at this stage in larvae of white Bream (*Diplodus sargus*), European seabass, red drum (*Sciaenops ocellatus*) and Senegalese sole (Cara et al., 2003; Zambonino Infante and Cahu, 2001; Navarro-Guilén et al., 2017). It is obvious that larvae have a different digestive capacity, both respect to juveniles and adults; nevertheless, the application of microfeeds in larvae feeding reflects their capacity to ingest and digest inert feed at first feeding (Cahu et al., 2003; Gawlicka et al., 2000; Lazo et al., 2000; Oozeki and Bailey, 1995; Yúfera et al., 2005).

1.4 Curcumin in fish diet

Today academia and industry are both interested on plant natural products and plant secondary metabolites, mainly due to their wide applications in humans' life. In fact, they can be used as dietary supplements or therapeutic formulation, dyes and ingredients in cosmetic industry. They can also been applyed as flavouring, growth promoter, antioxidant and immunomodulatory agents in animal industry (Aggarwal and Sung, 2009; Al-Sagheer et al., 2018; Greathead, 2003; Srivastava et al., 2011). In fact, being modern animal farming mostly characterized by intensive rearing conditions, animals are subjected to abiotic and biotic stress-generator factors that may have negative consequences on animal growth rate, zootechnical performance and on immune system defense (Lieke et al., 2020; Nya and Austin, 2011; Wang et al., 2015; Yin et al., 2009). In order to alleviate these negative effects, different studies investigated the zootechnical performance of reared animals fed on diet supplemented with plant derived products (Hosseini-Vashan et al., 2020; Ibrahim et al., 2019; Magouz et al., 2020; Ran et al., 2016; Türk et al., 2016). Among these

products currently under inquiry, curcumin is largely used in feed animal of zootechnical/economical value, such as cows (Vorlaphim, 2011), pigs (Ilsley et al., 2005), poultry (Guil-Guerrero et al., 2017) and fish (Baldissera et al., 2018)

Curcumin is a yellow coloring agent active polyphenol present in the rhizome of the spice turmeric plant (*Curcuma longa* L.), and native to Southeast Asia. Curcumin has been part of Asian medicine for centuries since the time of Ayurveda (1900 BC) and nowadays it is use also in Western medicine in particular as anti-cancer agent (Abrahams et al., 2019; Avanço et al., 2017; Buhrmann et al., 2020). In laboratory conditions, curcumin showed in fact numerous therapeutic activities such as antioxidant, inhibiting highly toxic reactive oxygen species (ROS), antiinflammatory, antibacterial, anticancerous, antiviral, antistress, immunomodulatory, free-radical-scavengers and digestive activity promoter (Baldissera et al., 2018; Bellio et al., 2014; Farhangi et al., 2015; Mandal et al., 2009; Mouler Rechtman et al., 2010; Prasad et al., 2014; Shi et al., 2015; Srivastava et al., 2011). Because of all the previously mentioned effects, the interest to introduce curcumin in animal diets is wide and well documented (Akdemir et al., 2017a).

In the aquaculture sector, most of the studies where curcumin was supplemented in the diet involved adult or at least juvenile stages, while almost no studies have been performed using larvae. In rainbow trout for example, curcumin was supplemented as dietary additive in order to alleviate the adverse effects of high stock density; it was proved that 200 mg of curcumin per kg of diet promoted higher body weight, feed intake and weight gain (Akdemir et al., 2017a). Another study with crucian carp (*Carassius auratus*) demonstrated that growth performance, digestive enzyme activities and intestinal antioxidant capacity gave best results supplementing 5 g of curcumin per kg of diet (Jiang et al., 2016). In tilapia (*Oreochromis mossambicus*) curcumin was included in diet at 0.5-1% doses for 35 days resulting in a significant increase of α-amilase, protease and lipase activities and also of insulin-like growth factors such as IGF-1 and IGF-2 (Midhun et al., 2016). Juveniles of Wuchang bream (*Melagobrama amblycephala*) were fed for 60 days with different concentrations of curcumin in the diet, the treatment with 60 mg of curcumin per kg showed not only better growth performance like, weight gain rate, specific growth rate and lower feed conversion rate, but also improved the non-specific immune response (Ge et al., 2015). Juvenile of Nile tilapia (*Oreochromis niloticus*) fed on a curcumin inclusion of 150 mg per kg of feed showed also better growth rate, antioxidant capacity and resistance to infection (Cui et al., 2013). Despite the good results in terms of growth rate, antioxidant status and immune responses, further research are strongly encouraged, in particular trying to apply this natural component into fish larvae microdiets.

In general, the antioxidant capacity of a substance is related to its reducing power which implies the ability of the sample to donate an electron and interfere with the free radical chain reaction (Priya et al., 2012). The presence and the production of antioxidants is therefore of vital importance, being able to keep under control the level of oxidative agents with the final task of neutralizing/inhibiting their deleterious effects through the modulation of some important cellular molecules (Ahmad et al., 2000). These cellular molecules are known as transcription factors and they act as a corrector against impairment of transcription, translation, oxidative metabolism, RNA processing, membrane structures and fuction at cellular level (Vaquerizas et al., 2009).

In this study the effects of dietary curcumin as growth promoter and antioxidant agent were investigated on marine fish larvae. Gilthead seabream larvae were fed on inert diet with different curcumin inclusion levels since mouth opening. Survival rate, growth rate performance, feeding incidence, digestive enzyme activities and antioxidant status were analyzed.

2. Materials and methods

2.1 Experimental objective

The objective of this work was to promote fish larvae robustness by combining metabolic programming and early nutrition concepts. For that, the effects of curcumin through exogenous feeding since mouth opening as modulator of gut maturation and antioxidant status in first larvae was evaluated.

Key performance indicators, gut maturation, feeding incidence and antioxidant status of fish larvae fed on two different dietary curcumin supplementation levels (Low and High) was analyzed and compared with larvae fed on a commercial diet (Control). Samplings were performed at three different developmental stages: 10 DAH (LA), 24 DAH (WN) and 31 DAH (END).

The experiment was carried out in compliance with the Guidelines of the European Union Council (Directive 2010/63/EU) and Portuguese legislation for the use of laboratory animals, with the approval of the CCMAR-CBMR ORBEA Animal Welfare Committee for the project PROLAR – Early metabolic programming in fish through nutritional modulation, (ref. ALG-01-0145-FEDER-029151). CCMAR facilities and their staff are certified to house and conduct experiments with live animals (licensed by the 'Direção Geral de Alimentação e Veterinaria', Ministry of Agriculture, Rural Development and Fisheries of Portugal).

2.2 Rearing conditions

Gilthead seabream larvae of 4 DAH were supplied by the Laboratory of Marine Cultures at the University of Marine and Environmental Sciences (Puerto Real, Cádiz, Spain) with an initial individual dry weight of 0.03 ± 0.005 mg larva^{-1} and transferred to Ramalhete Marine Station (Universidade do Algarve, Faro, Portugal).

Larvae were equally distributed in 9 cylindro-conical tanks (100 L) in a semi-closed recirculation system with an initial density of 284 larvae L^{-1} (28400 larvae/tank). The experimental system was equipped with a mechanical filter, a submerged biological filter, a protein skimmer and a UV sterilizer. The water parameters were maintained as follow (means ± SD): temperature 19.2 ± 0.02 ºC, salinity 36.3 ± 0.6 g L^{-1} and dissolved oxygen in water 93.8 ± 0.4% of saturation. Photoperiod was 10 light :14 dark. A daily monitoring of environmental parameters and larval mortality was performed; the rearing tanks were cleaned regularly to preserve water quality.

2.3 Experimental design and feeding plan

Three treatments were randomly assigned to 9 tanks: CTRL – commercial diet; LOW – low dietary curcumin supplementation level and HIGH – high dietary curcumin supplementation level. Inert diets (commercial and experimentals) were manufactured by SPAROS Lda (Olhão, Portugal). Curcumin inclusion level in HIGH diet was the double respect to LOW diet, while no curcumin was present in diet of treatment CTRL. Each treatment was performed in triplicate.

Larvae from all treatments were fed according to the feeding plan based on rotifers (*Brachionus plicantilis*) enriched with DHA protein Selco (Inve, Belgium), *Artemia* nauplii (Inve, Belgium) and inert diet. The amount of feed offered, co-feeding intervals and inert diet size were the same for all treatments and dependent on larval age according to the following protocol: at mouth opening (4 DAH) larvae were fed on rotifers in co-feeding with inert diet. Rotifers were initially increased from 12 rotifers mL^{-1} to 16 rots mL^{-1} and then progressively reduced until 13 DAH. *Artemia* nauplii was introduced at an initial density of 0.3 AF mL^{-1} at 10 DAH and then gradually reduced until 0.15 AF mL^{-1} at 24 DAH (Weaning). Inert diet was offered from 4 DAH to the end (31 DAH). The diet was initially supplied at a commercial size of 100-200 µm from 4 to 11 DAH, then at an equal mixture (50/50) of 100-200 µm / 200-400 from 12 to 24 DAH and finally it was supplied a size of 200-400 µm until the end of the experimental period (31 DAH). Total daily amount of inert diet was divided in five meals per day while the total amount of live preys was initially divided in three meals (4-9 DAH) and later reduced to two times per day (10-23 DAH). Larval rearing system was based on green water technique using *Nannochloropsis oculata* (4-23 DAH) (figure 2.3.1).

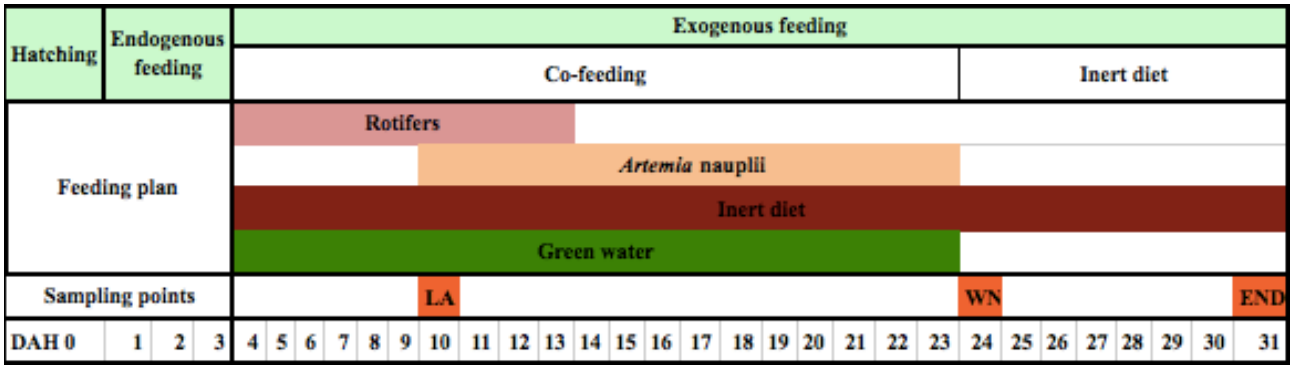


Figure 2.3.1. Schematic drawing of experimental feeding regime and sampling times of *S. aurata* larvae during the experiment (4 to 31 DAH).

2.4 Key performance indicators

Survival rate (%) for each treatment was determined at the end of the experiment (31 DAH) by direct counting of individuals, considering the intermediate samplings, by the formula in (1):

(1) Survival (%) = (final fish number / initial fish number) x 100

Growth performance at each sampling point was assessed by individual dry weight (DW) and total length (TL) measurements (*n* = 15 per replicate, except for larvae at 4 DAH that were 30 pooled-larvae, 3 pools/treatment). Dry weight measurements were obtained from freeze-dried samples using a high precision microbalance (± 0.001 mg; MSA36S-000-DH, Sartorius, Germany); previously, larvae were washed twice in distilled water and snap-freeze in liquid nitrogen. Total length was performed using the Leica Application Suite LAS (Leica Microsystems, Germany) for digital image analysis. The same larvae were used for dry weight and total length in order to calculate larval condition factor (K). Based on those parameters the condition factor was calculated according to Fulton's condition factor formula in (2) (Mozsár et al., 2015).

(2) K= final body weight (mg)/[final body length (mm)]^3

Individual growth was also valuated measuring the relative growth rate (RGR, % day $^{-1}$) following the formula in (3) (Ricker and Parker, 1960):

(3) $RGR = (e^{g} - 1) * 100$, where $g = [(\ln_{\text{final weight}} - \ln_{\text{initial weight}}) / \text{time}]$

2.5 Gut maturation

Gut maturation was evaluated through the analysis of digestive enzyme activities, such as trypsin, chymotrypsin, aminopeptidase-N, amylase, lipase and alkaline phosphatase activities. Larval sampling per sampling point and treatment was as follows: LA: 4 pooled-larvae (n = 9), WN: 3 pooled-larvae (n = 15), END: 2-3 pooled-larvae (n = 6). Samples were freeze-dried and manually homogenized in 250 μL (LA), 230 μL (WN) and 350 μL (END) of distilled water. The homogenate was centrifuged for 5 min at 12.500 g, 4°C to remove the tissue, and the enzymatic extract (supernatant) was used for the analysis. All samples were kept in ice during the process described above to avoid enzyme denaturation and /or damage. Enzyme extracts were kept at -20°C until analysis.

For proteases activity measurement, trypsin, chymotrypsin and aminopeptidase-N, the fluorogenic substrates Boc-Gln-Ala-Arg-7- methylcoumarin hydrochloride (BOC, Sigma-Aldrich B4153), N-Succinyl-Ala-Ala-Phe-7-amido-4-methylcoumarin (Sigma-Aldrich S8758) and Nα-

Benzoyl-L-arginine-7-amido-4-methylcoumarin hydrochloride (Sigma-Aldrich B7260), respectively, were diluted in dimethyl sulfoxide (DMSO) to a final concentration of 20 μM. For analysis, 5 μL of substrate, 190 μL of 50 mM Tris + 10 mM $CaCl_2$ buffer (pH 8.5, without $CaCl_2$ for aminopeptidase) and 15 μL of the larval homogenate were added to the microplate (Sanz and Toldrá, 2002; Rotllant et al., 2008). Fluorescence was measured at 355 nm (excitation) and 460 nm (emission).

Ultra Amylase Assay Kit (E33651) from Molecular Probes was used for amylase analysis. This kit contains a starch derivate labeled with a fluorophore dye as substrate. This substrate was diluted in substrate solvent (sodium acetate; pH 4.0) and reaction buffer (0.5 M MOPS; pH 6.9) and, to a final concentration of 200 μg/mL. For analysis, 50 μL of the substrate solution and 15 μL of the larvae extract were added to the microplate. Fluorescence was measured at 485 nm (excitation) and 538 nm (emission).

Lipase activities were assayed using 4-methylumbelliferyl butyrate (Sigma-Aldrich 19362), and 4-methylumbelliferyl oleate (Sigma-Aldrich 75164) as susbtrates for for 4-C and 18-C like lipases, respectively. Substrates were dissolved in phosphate buffer (pH 7.0) to a final concentration of 0.4 mM (modified method from Rotllant et al., 2008), aliquoted and stored at –20 °C. 15 μL of the larvae homogenate was added to the microplate and mixed with 250 μL of 0.4 mM substrate for the analysis. Fluorescence was measured at 355 nm (excitation) and 460 nm (emission).

For alkaline phosphatase analysis the substrate used was 4-Methylumbelliferyl phosphate disodium salt, (MUP, Sigma-Aldrich M8168). A 1 mmol/L stock solution of MUP was prepared by dissolving the substrate in borate buffer (pH 8). 15 μL of the enzymatic extract was added to the microplate and mixed with 100 μL of substrate for the analysis (modified from Fernley and Walker, 1965). Fluorescence was measured at 360 nm (excitation) and 440 nm (emission).

All enzyme activities were expressed as RFU (Relative Fluorescence Units) per mg larva dry weight.

2.6 Feeding incidence

To evaluate the feeding incidence (absence/presence of feed in the gut) 10 larvae per replicate were sampled at 5, 6, 8, 12, 16, 20, 23 and 28 DAH, always at 2.00 pm to ensure the same feeding status between sampling days. For gut content estimation, gut fullness level was examined by image analysis based on the technique described by Romero-Romero and Yúfera (2012) and Mata-Sotres et al. (2015). Larvae were photographed under the microscope connected to Leica Application Suite (LAS) for digital image analysis. The level of gut fullness was determined

measuring the pigmented area within the digestive cavity (figure 2.6.1). Gut content was normalized for larvae size using the ratio between the fullness area and the total length of each larvae. For feeding incidence estimation, per tank and sampling point, larvae with gut fullness lower than 10% respect to the maximum recorded were considered empty. The data analysis was performed using Image J software (National Institute of Health, Bethesda, MD) (Abramoff et al., 2006).

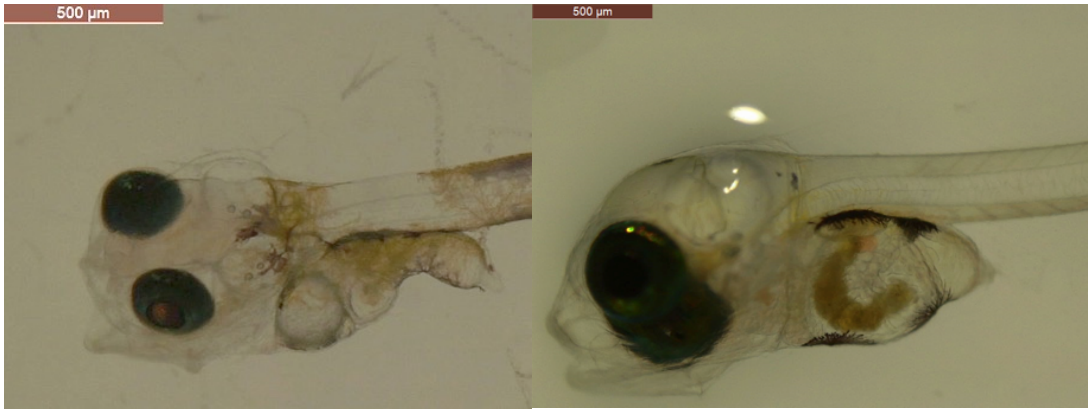


Figure 2.6.1. Example of 8 DAH (left) and 28 DAH (right) of *S. aurata* larvae used for feeding incidence.

2.7 Antioxidant status

The antioxidant status of the larvae was assessed by the measurement of the following oxidative stress biomarkers: total glutathione (TG), total antioxidant capacity (TAC), protein carbonylation (PC) and mitochondrial oxidative status (MOS). For the analyses 50 pooled larvae were collected at LA; 30 pooled larvae at WN, and 20 pooled larvae at END (n=1 per replicate, n=3 per treatment). Larvae was washed twice in distilled water and then snap-freeze in liquid nitrogen and stored at -80°C until being analized.

2.7.1 Sample preparation for biomarkers analysis

For the analysis of TG, TAC and PC, fish samples were homogenized using a tissuelyser (Star-Beater, VWR, USA) in 1200 µl of ultra-pure water. 700 µL of the supernatant was diluted in 0.2 M K-phosphate buffer (pH 7.4, vol. 1:1), and then centrifuged for 10 min at 10,000 g and 4 ºC. The post-mitochondrial supernatant (PMS) was then divided into three aliquots of 250 µL for TG, TAC and PC and 50 µL for Bradford analysis. The samples were kept on ice during the assay and the aliquots maintained at –80 ºC until further analyses.

For the analysis of MOS, fish samples were homogenized using a tissuelyser (Star-Beater, VWR, USA) in 1 mL of buffer containing 225 mM manitol, 75 mM sucrose, 1 mM EGTA and 4 mM HEPES (PH 7.2) following the protocol described by da Silva et al. (2015). The homogenate

was centrifugated for 10 min at 1,200 g and 4°C. The PMS was carefully removed and centrifugated again for 10 min, 16,500 g at 4°C. The pellet was re-suspended in a buffer containing 250 mM sucrose and 5 mM HEPES (PH 7.2). The volume of buffer utilized was 500 µL, 650 µL and 750 µL for LA, WN and END respectively. The samples were kept on ice during the assay and then maintained in –80 ºC until further analyses.

All biomarkers determinations were performed spectrophotometrically, in 96 well flat bottom microplates, with a temperature-controlled microplate reader (Synergy 4 BioTek, USA).

Protein concentration of PMS was determined according to the Bradford method (Bradford, 1976), using bovine γ-globulin as a standard.

2.7.2 Oxidative stress biomakers measurement

Total glutathione content (TG) was determined using a recycling reaction of reduced glutathione (GSH) with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) in the presence of glutathione reductase (GR) excess (Baker et al., 1990; Tietze, 1969). Briefly, 250 µL of a reaction buffer compossed by Na-K phosphate buffer, NADPH, DTNB and GR was mixed with 50 µL of sample in the microplate; kinetic was measured at 412nm during 3 min. TG content was calculated as the rate of TNB^{2-} formation with an extinction coefficient of DTNB chromophore formed, $\varepsilon = 14.1 \times 103 M^{-1} cm^{-1}$ (Baker et al., 1990; Rodrigues et al., 2017). Results were expressed in mmol GSH per mg protein.

Total antioxidant capacity (TAC) was assessed following the protocol described by Erel (2004), using colored 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation ($ABTS^{\cdot+}$). This method is based on the colorless molecule ABTS, which is oxidized to a characteristic blue-green $ABTS^{\cdot+}$. When the colored $ABTS^{\cdot+}$ is mixed with any substance that can be oxidized, it is reduced to its original colorless ABTS form again; in contrast, the reacted substance is oxidized. This change in color was measured as a change in absorbance at 660 nm and the assay was calibrated with Trolox. Briefly, 5 µl of sample was mixed in a microplate with 200 µl of acetate buffer solution (0,4 mol/l, pH 5.8) and 20 µl of $ABTS^{\cdot+}$ (in acetate buffer at 30 mmol/l, pH 3,6). The first reading was taken just after adding all the reagents (as sample blank) while the last absorbance was taken at the end of the incubation period (5 min after the mixing). Results were expressed in mmol Trolox equivalent per mg protein

Protein carbonylation (PC) was measured based on the reaction of 2,4-dinitrophenylhydrazine (DNPH) with carbonyl groups, according to the DNPH alkaline method described by Mesquita et al. (2014). 120 µL of DNPH (10 mM in 2M HCl) was added to 120 µL of sample and after 10 minutes of incubation, 60 µL of NaOH (6 M) was added. The amount of carbonyl groups was quantified spectrophotometrically at 450 nm after 10 minutes of incubation at

room temperature against a blank where the sample was substituted by an equal volume of buffer solution (22,308 $mM^{-1}cm^{-1}$extinction coefficient). Results were expressed in nmol carbonyl per mg protein

The mitochondrial reactive oxygen species was assessed by the dihydrodichloro-fluorescein diacetate –H(2)DCF-DA (da Silva et al., 2015; Van Der Toorn et al., 2007). This dye is non-fluorescent when chemically reduced, but after cellular oxidation and removal of acetate groups by cellular esterases it becomes fluorescent (Garcia-Ruiz et al., 1995). The mitochondrial suspension (0.5 mg protein) were incubated in the presence of 1 μM DCFDA and fluorescence was monitored over 5 min of gentle shaking at 28°C, with excitation and emission wavelengths of 503 and 529 nm, respectively. Under the described conditions the linear increment of fluorescence indicated the rate of ROS formation. Results are expressed as Relative Fluorescence Units (RFU) per mg mitochondrial protein.

2.8 Statistical analysis

Differences in growth performance, feeding incidence, digestive enzymes activities, antioxidant status parameters, and body proximal composition due to dietary treatments were evaluated using a one-way ANOVA after assessing equality of variances by a Levene's test. Post hoc multiple comparisons were carried out using Tukey's test. If equality of variances was not observed a Kruskal-Wallis non-parametric test was performed. To evaluate changes in enzymatic activity between ages, the enzymes detected only at WN and END sampling points were tested by means of an unpaired two-tailed Student's *t*-test. All percentage data were arcsine square root-transformed prior to analysis. Statistical significance was set at reliability level of 0.05 and all the results were reported as arithmetic mean values ± standard deviation (SD). SPSS 26.0 software was used for statistical analysis and graphs (IBM, USA) while charts were done with Excel (Excel for Mac, version 2011).

3. Results

3.1 Key performance indicators

Dietary treatments had no significant impact on survival rate. Survival rates at the end of the experiment (31 DAH) were 2.12 ± 0.63, 1.99 ± 0.59 and 1.54 ± 0.10%, CTRL, LOW and HIGH, respectively.

Growth performance parameters were similar between treatments at all sampling points (table 3.1.1). On average, individual dry weight increased from 0.02 ± 0.005 mg larva^{-1} at the beginning of the experiment (4 DAH) to 0.22 ± 0.01mg larva^{-1} at the end of the experiment (31 DAH). Results were close to being statistically different at 31 DAH (P=0.059), tending to be higher in the treatments with dietary curcumin supplementation.

Individual total length at the end of the experiment was 5.93 ± 0.05 mm larva^{-1}. There were no statistical differences between treatments though it was slightly higher in treatment LOW.

Condition factor doubled during the experiment. Although there were not statistical differences between treatments, like in dry weight results, it was close to being statistically different at 31 DAH (P=0.059), tending to be higher in the treatments with LOW dietary curcumin supplementation.

The relative growth rate (RGR) did not show significant differences between treatments along the experiment. Average RGR, considering all the experimental period, was 7.99 ± 0.75 % day^{-1}. When analysed by sampling intervals, average RGR were -5.45 ± 1.60, 22.49 ± 2.65 and 5.71 ± 3.49 % day^{-1}, for the intervals 4 – 10, 10 – 24 and 24 – 31 DAH, respectively.

Table 3.1.1. Growth performance parameters of *S. aurata* larvae: dry weight (DW), total length (TL) and condition factor (K) at 10 DAH (n = 45 per treatment), 24 DAH (n = 45 per treatment) and 31 DAH (n = 24 per treatment). Relative growth rate (RGR) was measured for each interval time (4-10, 10-24 and 24-31 DAH) and for the overall period of the experiment (4-31 DAH).

	Treatments								
	CTRL			LOW			HIGH		
DW									
10 DAH	0.02	±	0.005	0.02	±	0.006	0.018	±	0.005
24 DAH	0.15	±	0.075	0.15	±	0.081	0.15	±	0.07
31 DAH	0.21	±	0.082	0.24	±	0.11	0.22	±	0.089
TL									
10 DAH	3.56	±	0.25	3.61	±	0.15	3.57	±	0.14
24 DAH	5.5	±	0.69	5.31	±	0.65	5.65	±	0.66
31 DAH	5.91	±	0.62	6.03	±	0.57	5.86	±	0.67
K									
10 DAH	0.23	±	0.08	0.21	±	0.08	0.2	±	0.06
24 DAH	0.42	±	0.08	0.42	±	0.14	0.39	±	0.07
31 DAH	0.47	±	0.08	0.54	±	0.12	0.52	±	0.1
RGR									
4-10 DAH	- 4.71	±	0.89	- 4.97	±	2.42	- 6.8	±	0.46
10-24 DAH	22.12	±	2.93	22.01	±	3.75	23.34	±	1.96
24-31 DAH	5.04	±	5.66	6.76	±	3.28	5.31	±	1.55
4-31 DAH	7.87	±	0.5	8.23	±	0.95	7.87	±	0.98

Results are means ± SD. Absence of letters indicates no statiscal difference between treatments.

3.2 Gut maturation

Along the experiment, all the digestive enzymes analysed were detected at least in the most advanced stage of development (31 DAH, END). This suggests a regular development of the larvae digestive system in all the treatments.

3.2.1 Proteases

Trypsin activity showed an overall increment during the experimental period. In fact, after an initial decrease recorded between 10 and 24 DAH, it followed an increasing trend until 31 DAH, being this increment statistically significant for all treatments (P=0.000) (table 3.2.1, annexes). Trypsin activity at 24 DAH was really close to be statistically different between treatments (P=0.054), tending to be higher in larvae from HIGH treatment. This tendency became statistically significant at 31 DAH (P =0.002), with higher trypsin activity in larvae from treatment HIGH when compared with CTRL larvae (figure 3.2.1.a), and similar between larvae fed the curcumin supplemented diets (LOW and HIGH).

Chymotrypsin and aminopeptidase activities were detected at the end of the experiment (31 DAH). Chymotrypsin activity reached the highest value (P=0.004) in larvae from HIGH treatment. Aminopeptidase activity did not show statistical differences between treatments, although values tend to be lower in larvae from LOW treatment (figure 3.2.1 b and 3.2.1.c, respectively).

The trypsin:chymotrypsin ratio (T:C) did not reveal significant difference between treatments, although it was slightly higher in larvae fed on CTRL diet (table 3.2.1, annexes). Moreover, CTRL fish showed also a coefficient of variation clearly higher (17.77%) than LOW (8.64%) and HIGH (8.02%).

3.2.2 Amylase

Amylase activity was only detected in the older stages of development, at WN (24 DAH) and END (31 DAH), showing an overall tendency to decline. The reduction was statistically significant in larvae from CTRL and HIGH diets (P=0.026 and 0.002, respectively), decreasing almost in half from 24 to 31 DAH (table 3.2.1, annexes). Regarding differences in amylase activity between treatments, it was observed a pattern of higher activity in the CTRL treatment (without curcumin supplementation), however, without statistical differences (figure 3.2.1.d).

3.2.3 Lipase

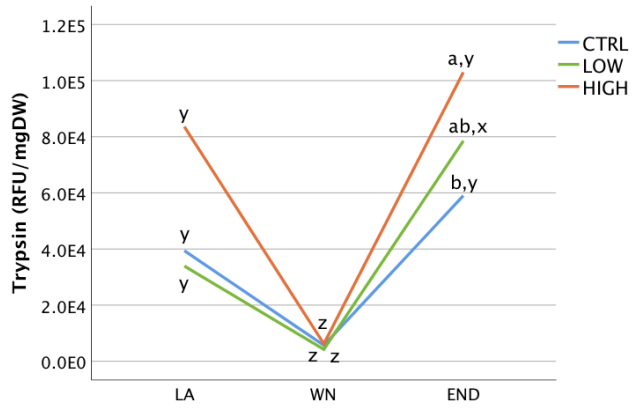
Both lipases showed decreasing patterns among sampling points. In particular 4-C like lipase activity, which was detected in all developmental stages, significantly decreased in all treatments between 10 and 24 DAH (P=0.000 for CTRL, LOW and HIGH) (table 3.2.1, annexes). At 10 DAH, activity was significantly higher in larvae from LOW respect to HIGH treatment (P=0.041). At 24 DAH, CTRL larvae showed the significantly highest activity (P=0.004); while at 31 DAH no statistical differences were detected between treatments (figure 3.2.1. e).
By contrast, 18-C like lipase activity was only detected at 24 and 31 DAH. In this span of time the enzymatic activity decreased significantly in all treatments (P=0.000, P=0.0024 and P=0.004 for CTRL, LOW and HIGH respectively) (table 3.2.1, annexes). Results showed also a significant difference at 24 DAH, with higher activity in CTRL larvae compared to LOW and HIGH treatments (P=0.000) (figure 3.2.1.f).

3.2.4 Alkaline phosphatase

Alkaline phosphatase activity was detected at 24 and 31 DAH; within this period the activity incremented, being statistically significant for all treatments (P=0.009, P=0.000 and P=0.022 for CTRL, LOW and HIGH respectively) (table 3.2.1, annexes). At 24 DAH, alkaline phosphatase activity was significantly higher in CTRL treatment compared to LOW treatment (P = 0.036).

Although a trend to lower values persisted in treatments with curcumin supplementation at 31 DAH, no statistical differences were recorded (figure 3.2.1.g).

A



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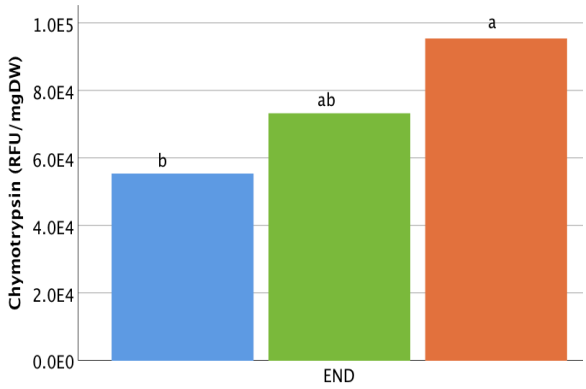

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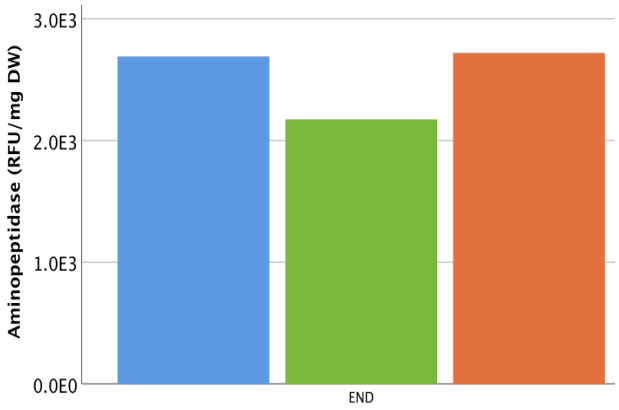

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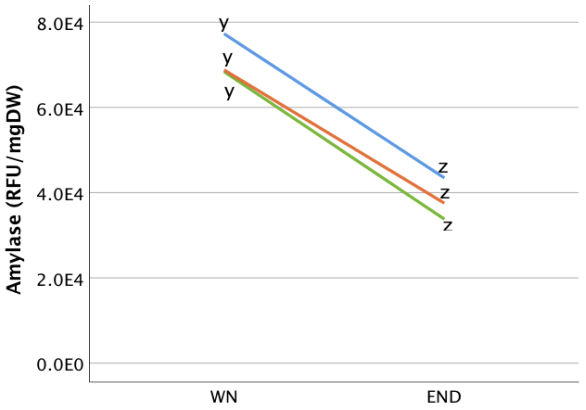

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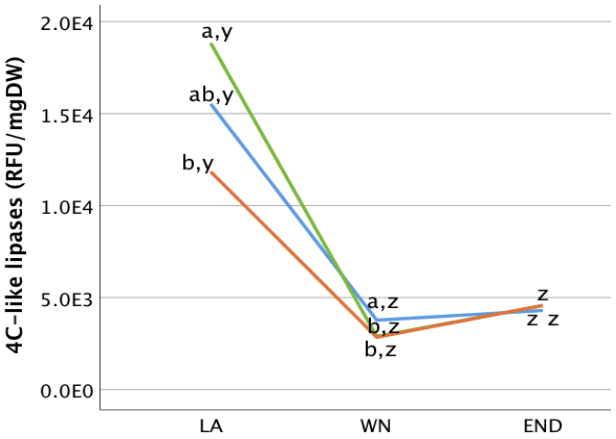

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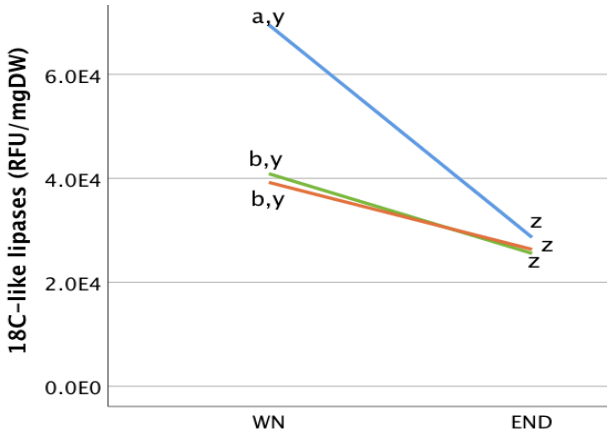

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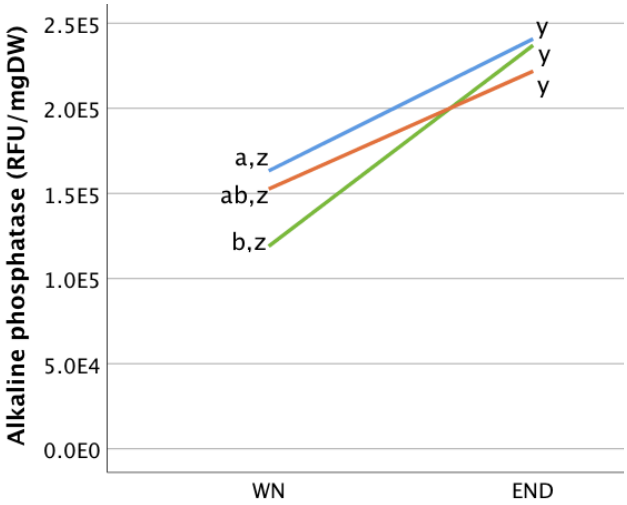

Figure 3.2.1. Enzymatic activity levels of trypsin (A), chymotrypsin (B), aminopeptidase (C), amylase (D), 4-C and 18-C like lipases (E and F, respectively) and alkaline phosphatase (G) (RFU/mg DW) in *S. aurata* larvae at different sampling points, respectively LA (10 DAH; 4 pooled-larvae), WN (24 DAH, 3 pooled-larvae) and END (31 DAH, 2-3 pooled-larvae). Blue, green and orange colors for CTRL, LOW and HIGH treatments, respectively (lengend shown on graph A). Different letters (x,y,z) represent significant differences inside each treatment during larvae development. Different letters (a,b) represent significant differences between treatments at the same larval age ($P<0.05$). Absence of letters indicates no statiscal difference between the treatments at the same larval age ($P>0.05$).

3.3 Feeding incidence

Treatments showed similar patterns of feeding incidence (presence/absence of feed in the gut) among all sampling points. CTRL larvae showed statistical differences between samplings, with the highest feeding incidence at 20 and 28 DAH and the lowest at 8 and 12 DAH ($P=0.007$). At 8 DAH larvae from CTRL and LOW treatments had a significantly higher feeding incidence than HIGH ($P=0.027$) (figure 3.3.1). Along the experiment more that 50% of larvae in CTRL and LOW treatments were detected with feed in the gut, with the only exception of LOW at 6 DAH. HIGH treatment generally showed lower values, below the 50% at 8 and 12 DAH, and generally higher variability along the experiment. Since the introduction of *Artemia* nauplii (10 DAH) a higher percentage of larvae have been detected with feed in the gut in all the treatments. Moreover, the transition between co-feeding and inert diet (24 DAH) had not negatively influenced the feeding incidence that remained stable between 23-28 DAH, with almost 80% of the larvae presenting feed in the gut.

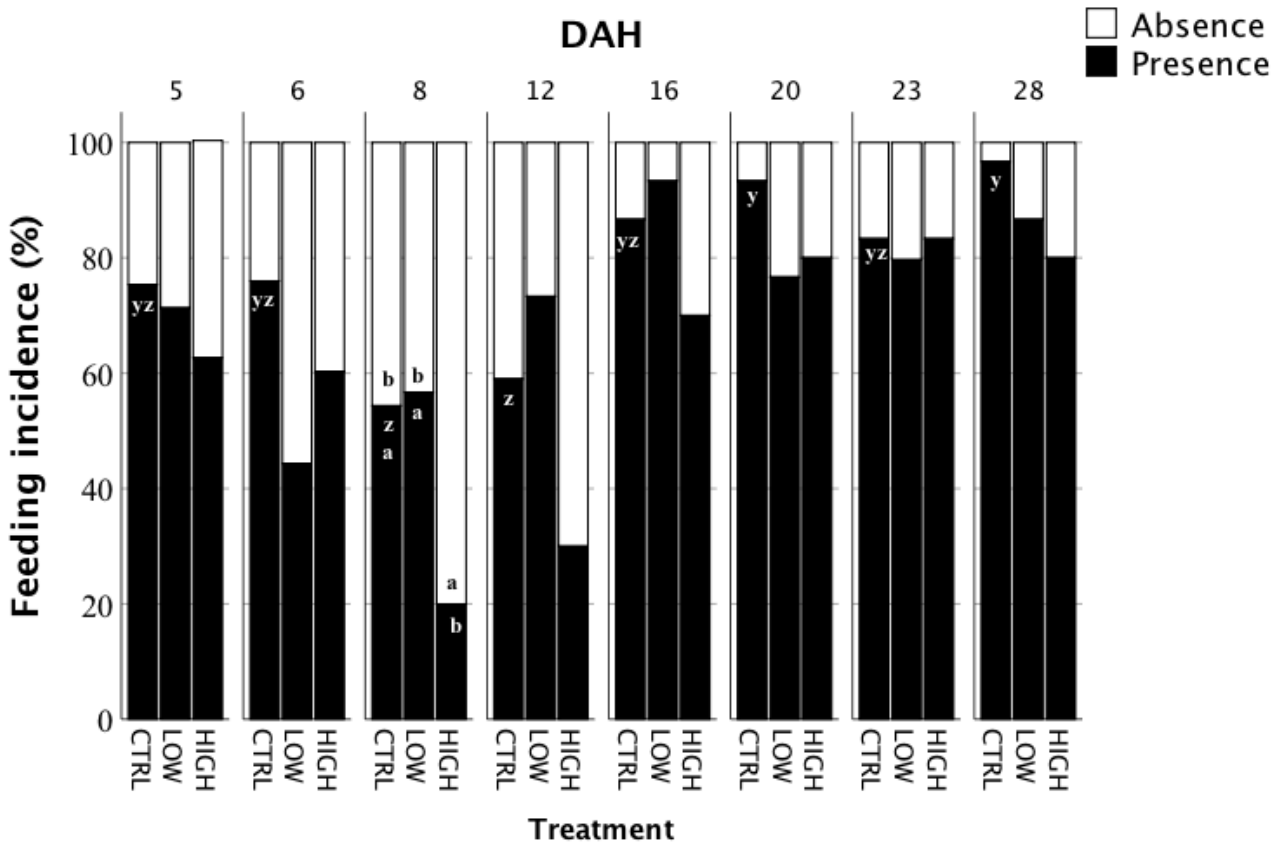

Figure 3.3.1. Feeding incidence (%) of *S. aurata* larvae at 5, 6, 8, 12, 16, 20, 23 and 28 DAH (n=30 per treatment). Different letters (y,z) represent significant differences inside each treatment during larvae development. Different letters (a,b) represent significant differences between treatments at the same larval age ($P<0.05$). Absence of letters indicates no statiscal differences ($P>0.05$).

Gut fullness revealed statistical differences along the larval development. An increase in gut fullness was recorded from 12 DAH – onwards for all the treatments (P=0.00, 0.000 and 0.000 for CTRL, LOW and HIGH, respectively) (figure 3.3.2). For what concern differences between treatments at the same larval age, results showed significant differences only at 12 and 23 DAH (P=0.028 and 0.001, respectively), where higher gut fullness levels were observed in larvae from treatment HIGH compared to CTRL and LOW (table 3.3.1, annexes).

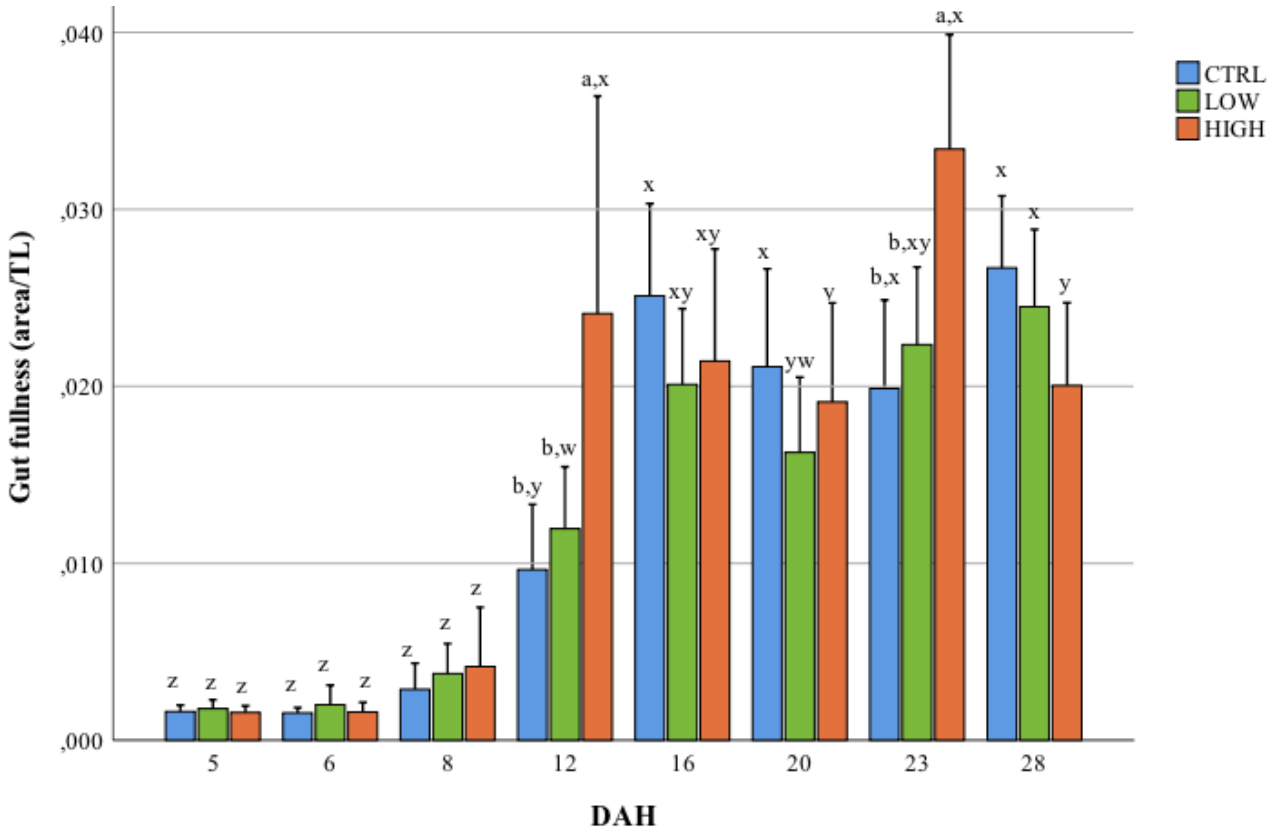

Figure 3.3.2. Gut fullness (area / TL) of *S. aurata* larvae at 5, 6, 8, 12, 16, 20, 23, 28 DAH (n=30 per treatment). Blue, green and orange colors for CTRL, LOW and HIGH treatments, respectively. Different letters (x,y,w,z) represent significant differences inside each treatment during larvae development. Different letters (a,b) represent significant differences between treatments at the same larval age ($P<0.05$). Absence of letters indicates no statiscal difference between the treatments at the same larval age ($P>0.05$).

3.4 Antioxidant status

Antioxidant status biomarkers were detected in all sampling points.

3.4.1 Total glutathione content

Larvae total gluthatione content (TG) showed an overall increment along the experimental period. Although this increasing tendency was registered for all treatments, it was statistically significant for HIGH larvae (P=0.022). No differences between treatments at the same age were observed (figure 3.4.1.a).

3.4.2 Total antioxidant capacity

In general, TAC levels showed a decrease between LA (10 DAH) and WN (24 DAH) period followed by a slight increment in the subsequent period (WN-END). This was statistically different for CTRL and LOW fish (P=0.021 and P=0.035 respectively), while no differences were found for HIGH treatment (P=0.086). No differences were reported between treatments at the same age (figure 3.4.1.b).

3.4.3 Protein carbonylation

No statistical differences were observed for protein carbonylation neither between treatments at the same age nor ontogenetic differences. Overall, values tended to keep stable for CTRL and LOW treatments along the experiment, while for HIGH levels trended to increase between LA and WN sampling points, followed by a trend to decrease until the end of the experiment. PC levels in HIGH larvae were close to be statistically different between sampling points (P=0.058). No differences were observed between treatments at each sampling point (figure 3.4.1.c)

3.4.5 Mitochondrial oxidative status

Larvae of the different treatments showed a global tendency to increase their mitochondrial oxidative status along the experiment. This increment was only significant in CTRL treatment (P=0.000), increasing from 58.08±40.25 to 810.19±144.71 RFU/mg mitochondrial protein, no statistical difference was observed in LOW and HIGH fish (P=0.061 and P=0.077, respectively). No differences were detected between treatments at the same age (figure 3.4.1.d).

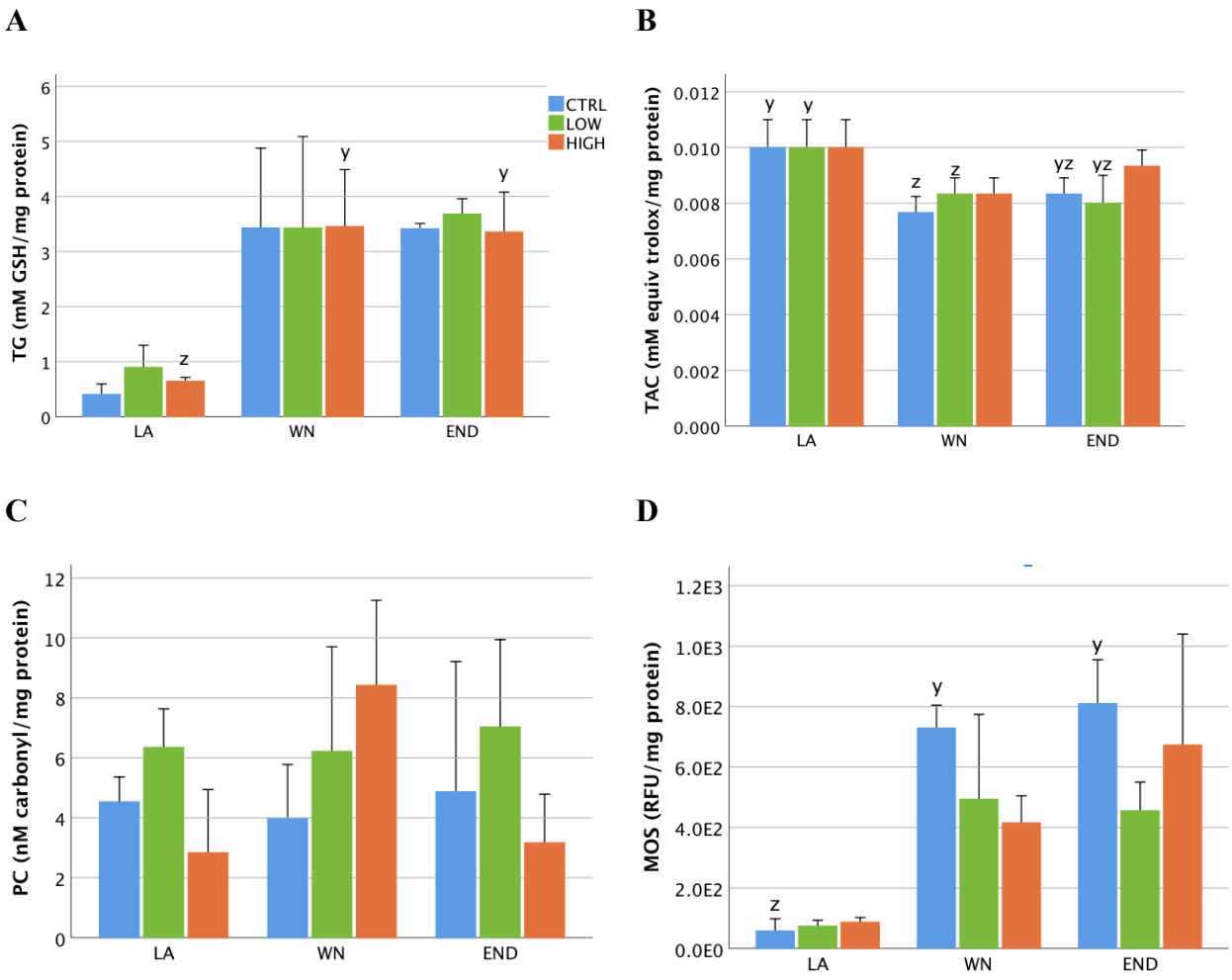

Figure 3.4.1. Antioxidant status biomarkers; total glutathione content (TG, A), total antioxidant capacity (TAC, B), protein carbonylation (PC, C) and mitochondrial oxidative status (MOS, D), of *S. aurata* larvae at 10 DAH (n = 50 pooled larvae per treatment), 24 DAH (n = 30 pooled larvae per treatment) and 31 DAH (n = 20 pooled larvae per treatment). Blue, green and orange for CTRL, LOW and HIGH treatments, respectively (legend shown in graph A). Different letters (y,z) represent significant differences inside each treatment during larvae development (P>0.05). Absence of letters indicates no statiscal difference inside each treatment during larval ages.

4. Discussion

Curcumin, a hydrophobic polyphenol extracted from the rhizome of tumeric (*Curcuma longa* L.), is largely known and investigated because of its wide range of pharmacological effects, such as: anti-inflammatory, anti-oxidant, anti-tumor, anti-bacterial, anti-viral, promoter of digestive capacity and immunomodulator (Akdemir et al., 2017b; Ming et al., 2019). The objective of this work was to investigate the effect of dietary curcumin both as early digestive promoter, fostering the ontogeny of the digestive system and the correlated enzymatic activity, and as antioxidant agent, attenuating the harmful effects of the oxidant molecules. Since mouth opening (4 DAH), gilthead seabream larvae were fed on microdiet with different curcumin concentrations (0, LOW and HIGH, respectively for CTRL, LOW and HIGH treatments), in co-feeding regime with live preys. At 24 DAH larvae were weaned and fed exclusively on inert feed until 31 DAH. Survival rate, growth rate parameters, feeding incidence, digestive enzyme activities and antioxidant status of the larvae from the different treatments were analysed and compared.

Among the factors limiting the success of the larval rearing process, one of the most important is related to larval nutritional requirements and digestive physiology (Hamre et al., 2013; Rocha et al., 2016; Rønnestad et al., 2013). With these implied restrictions, this study was facing a current huge challenge in the marine larviculture sector: the early substitution of live preys with inert diet. In fact, if survival rate and growth performance in the present study are compared to other trials in which inert diet, at the earliest, was supplied in co-feeding in larvae of 14 DAH onward, as in Costa, (2012), Pantazis et al., (2014), Perera and Yúfera, (2017), both parameters performed lower values. However, being this comparison certainly biased, due to the different rearing condition and especially due to differences in the feeding protocol, in particular considering that in this trial micro feed was provided since mouth opening at 4 DAH, further research need to be conducted. In addition, in this trial no differences were detected between treatments both, for survival and growth performance; thus, results leads to confirm that curcumin did not negatively influence both parameters and the results obtained should be justified by biological reasons, such as larval quality. Despite this, larvae fed on low curcumin supplementation (LOW diet) showed slightly better results, being close to the significance level ($P = 0.059$) both for weight and condition factor, especially when compared to CTRL larvae. This might suggest a positive effect of curcumin on growth performance. The RGR was also slightly higher in LOW treatment, but no significant differences were denoted. RGR measured by intervals reflected the effects of the different phases of the feeding plan on the larval growth. RGR was negative during the period 4 – 10 DAH in all the treatments while it showed a sharp increase in the following period (10 – 24 DAH), decreasing again after weaning (24 – 31 DAH). This might denote a possible negative correlation of RGR with

the feeding plan here utilized, that not always might have matched the nutritional requirements of the young larvae, especially during the period of co-feeding with rotifers. By contrast, the better growth performance was observed in the period of co-feeding with *Artemia*.

In general, few researchs have been conducted on fish fed on curcumin but trials performed with other animals tend to validate its positive effect on growth. For example, growing Japanese quails (*Coturnix japonica*) of 26.1 ± 0.08 g live weight, returned best growth rate performance when fed on curcumin inclusion diets (Reda et al., 2020); while the same positive effect was measured also in 15 days-old nursing Lacaune lambs (*Ovis aries*) of 5.34 ± 0.42 kg live weight, for which the highest growth rate was measured in treatment with curcumin in the diet (Molosse et al., 2019). These results suggest that curcumin might offer better results in term of growth; nevertheless larviculture will need future research to validate the effects of curcumin as survival and growth promoter.

Digestive enzymes analyses are of particular interest in order to evaluate gut maturation; indeed the digestion rate in the gut limits the uptake of nutrient and can potentially limit the growth rate of the whole organism (Lemieux et al., 1999; Rønnestad et al., 2013). In addition, if analyses on enzyme activities show a response to environmental changes, such as feeding regime or light cycle, the utilization of their values as gut maturation indicators is even more justified (Navarro-Guillén et al., 2015). The digestive enzymes under study were all detected along the experiment, at least at the older stages of development according to the species ontogenesis. There were impacts of the dietary treatments in larvae with the same age, and also impacts of the dietary treatments in the larval development. Overall, in all sampling points, it was noted a huge variability of the results. It is important to mention that all samplings were performed at the same time (9.00 am) and feeding condition to avoid differences between sampling points due to the feeding status of the larvae.

Fish larvae, being the vertebrate organism with the highest growth potential (up to 100%/day) (Conceição et al., 1998), require a protein- and lipid-rich feed in order to support the high energy requirements essential for fast growth (Hamre et al., 2013; Rønnestad et al., 2013). Intestinal proteases have a specific hydrolytic activity towards the protein/polypeptide chains; their precursors are mainly produced in the pancreas. Trypsin, generally accepted to be the most important proteolytic enzyme in the early stage of marine fish larvae, is among the pancreatic-proteolytic enzymes expressed since the first feeding (Cara et al., 2003; Rønnestad et al., 2013; Zambonino Infante and Cahu, 2007). Also, if it is not clear yet the reason behind the slightly tryptic activity reduction after the onset of the first feeding (Rønnestad et al., 2013) during the first three weeks of life, at least for temperate fish species, the secretion of this enzyme assumes a general growing trend (Cahu and Zambonino Infante, 2001). According to this, larval tryptic activity in the present study was detected along the whole experiment, following a decreasing and increasing trend, for the first (10-24 DAH) and second experimental periods (24-31 DAH), respectively.

Moreover, dietary curcumin seemed to have a positive effect on trypsin activity; in fact, along the whole experiment trypsin activity tended to be higher in HIGH larvae if compared to both, CTRL and LOW fish. This result is similar to the positive effect of curcumin on trypsin activation showed in juveniles of Crucian carp (*Carassius auratus*) and rats (Jiang et al., 2016; Platel and Srinivasan, 2000). Chymotrypsin, a proteolytic enzyme largely correlated to the maturation of the digestive system (Rønnestad et al., 2013), in this study was only measured at 31 DAH, with a strong difference between treatments. The highest chymotrypsin activity was detected in larvae fed on HIGH diet, meaning that supplying diet with curcumin inclusion promote the development of the larvae digestive capacity. In line to this, in a previous study by Mata-Sotres et al. (2016) with seabream larvae, despite chymotrypsin gene expression was recorded since 10 DAH, its activity was not detected during the whole experiment (until 60 DAH). This might suggest that curcumin can affect the post-translational regulation of this enzyme. In addition, some authors suggest to use also the trypsin: chymotrypsin ratio (T:C) as additional parameter to determine nutritional status both for fish larvae and adults (Cara et al., 2007; Rungruangsak-Torrissen et al., 2006; Sunde et al., 2001). This ratio indicates the extent of chymotrypsin activated by trypsin, therefore signaling the proteolytic capacity of the fish. Those authors also suggested that a higher T:C, indicates a higher absorption and transport rates of essential amino acids for protein synthesis. One limitation of this approach is represented by the opposite activity patterns of the enzymes; in fact, while trypsin usually decrease after the onset of a functional stomach, with gastric digestion settled, chymotrypsin continue to increase (Rønnestad et al., 2013). In this study T:C ratio was substantially equal between treatments, but a higher homogeneity was detected in treatments with curcumin supplementation, mostly due to a lower variability in chymotrypsin activity results. So, curcumin might have played a role as synchronyzer of the intestinal maturation of larvae from curcumin treatments (LOW and HIGH diets). Among the proteolytic enzymes studied in this work, aminopeptidase, contrary to trypsin and chymotrypsin, is produced by the intestinal brush border membrane (Cara et al., 2003; Zambonino Infante and Cahu, 2007). In marine fish larvae such as European seabass, red drum (*Sciaenops ocellatus)* and Senegalese sole, its level tends to increase since the fourth week after hatching onwards, characterizing the normal maturation of the enterocytes, this is not only characteristic of developing fish larvae but also of other species including mammals (Cahu and Infante, 1995; Kotzamanis et al., 2007; Rønnestad et al., 2013; Zambonino Infante and Cahu, 2001). In line with these studies, also in this trial aminopeptidase was only observed at 31 DAH, in concordance with the expected ontogeny of the larvae gut maturation. Aminopeptidase activity levels were similar between larvae from different treatments, especially between CTRL and HIGH diet while it was slightly lower in larvae fed on LOW diet. As observed for chymotrypsin, curcumin inclusion might have helped in the enzyme functionality, with lower

variability in larvae fed on dietary curcumin. This leads to suggest that curcumin might stimulate larvae gut development and promoting digestive capacity. In the end, curcumin seems to have played a positive role on proteolytic enzymes promoting the enzymes functionality.

Is it well recognized that the ability of fish to digest carbohydrates (mono-, di and polysaccharides) differs according to the feeding habits, from herbivorous to strict carnivorous, of different species (Rønnestad et al., 2013). Generally, carnivorous species do not use dietary carbohydrates as primary energy substrate; hence carbohydrase enzymes such as α-amylase, a fundamental enzyme for the digestion of high complex carbohydrates, have been less investigated especially if compared to proteolytic or lipolytic enzymes (Rocha et al., 2016). Despite this, there are several studies where amylase was detected since the early stages of life (Cahu and Infante, 1994; Cara et al., 2003; Gisbert et al., 2009; Ma et al., 2005; Moyano et al., 1996; Suzer et al., 2007), with clear different patterns between marine fish larvae species (Moyano et al., 1996; Naz, 2009; Zambonino-Infante et al., 2008). In those studies, it was reported that α -amylase is entirely synthetized by the pancreas, and activity was detected since the pancreas starts to be functional during the yolksac stage. Activity tends then to reach its peak after the first feeding followed by a decreasing pattern, this pattern is always species-specific (Rønnestad et al., 2013). In fact, while in herbivorous or omnivorous species the activity of amylase increases along the development (Zouiten et al., 2008), the opposite occurs in carnivorous species where, with the activation of the stomach, amylase activity tend to decrease (Cara et al., 2003; Zambonino-Infante et al., 2008). In this study, amylase was not detected until 24 DAH; this was in line with (Mata-Sotres et al., 2016) where amylase was detected for the first time in gilthead seabream larvae at 30 DAH, 0.187 ± 0.021 mg live weight, slightly smaller than larvae in the present trial. The same pattern was observed for Senegalese sole larvae, were amylase activity was measured only at 20 and 32 DAH (Navarro-Guillén et al., 2015). In the present study, at 24 and 31 DAH amylase, tended to be higher in larvae fed on commercial diet, but statistical differences were not found between the treatments. A declining pattern of amylase activity was observed in all the treatments, as similarly showed in Péres et al., (1996) and Ribeiro et al., (1999), confirming that young larvae tend to have a relative higher activity of amylase respect to the older. In conclusion, amylase activity decreased similarly in all treatments suggesting that early introduction of diet in a co-feeding regime had no detrimental effect on larvae digestive capacity.

Larvae dietary lipid utilization is a topic that has received an increasing attention during the last decades (Izquierdo et al., 2000a; Rønnestad et al., 2013; Zambonino Infante and Cahu, 2001). In fact, lipolytic enzymes play an important role in the digestion process of the young larvae due to the high energy needs in the early stages of life (Hamre et al., 2013); despite this, the estimation of

the marine fish larval capacity to digest lipids is still difficult (Rønnestad et al., 2013). In this study 4C-like lipase, able to digest short-lipidic chains, was detected since the beginning of the experiment and showed a decreasing pattern probably due to the substitution with lipolytic enzymes with affinity for more complex substrates, like 18C-like lipase, as indicated in Izquierdo et al., (2000). 4C-like lipase activity was significant different for all treatments between 10 and 24 DAH, confirming the main idea of the higher energy requirement at early stages; in fact at 10 DAH it was achieved the highest activity level for all treatments. Finally, at 31 DAH there were not differences between treatments; at this age in fact, activity levels were in the same range than at 24 DAH for all treatments. As observed for 4C-like lipase, also 18C-like lipase activity followed an unexpected decreasing tendency, contrary to what suggested in Mata-Sotres et al., (2016) where total lipase activity tend to increase from 10 to 30 DAH. Despite this, the dietary treatments did not had a negative role on lipases activity since there were not differences comparing to larvae fed on the commercial diet; in fact, the decreasing pattern of 18C-like lipase activity was significant in all the treatments. It is interesting to note in the end how, despite at 24 DAH the levels of both the lipases analyzed were lower in treatments with curcumin supplementation, larvae fed on curcumin diets were not significantly smaller, neither in terms of dry weight or total length. This could be derived due to the higher trypsin activity measured on these treatments. This opposite trend between trypsin and lipase enzymes activity was similar to what reported in a study with gilthead seabream larvae fed on different rehydrated microdiets (Yúfera et al., 2016). Nevertheless, further studies are suggested in order to better investigate both the functionality and the efficiency of lipolytic enzymes in larvae fed on curcumin diet.

Over the past years the intestinal isoform of alkaline phosphatase (IAP), among the major homeostatic enzymes produced by the enterocytes, has gained an increasing attention due to its capacity to keep inflammation under control and, as a consequence, to maintain a gastrointestinal and systemic health (Bilski et al., 2017; Lallès, 2019, 2014, 2010; Rader, 2017). The activity of alkaline phosphatase, that is related to the transport mechanism of extracellular digestion, is utilized as an marker to indicate a fully functionality and integrity of the intestinal epithelium (Novelli et al., 2016; Parma et al., 2020; Rønnestad et al., 2013; Toledo-Cuevas et al., 2011). Most of the publications reviewed by Lallès, (2019) reported that the highest IAP activity was localized in the proximal intestine, intermediate values in the mid-intestine while the lowest values were detected in the distal intestine, in both juvenile and adult fish. However, other studies reported that age is not the only factor affecting the functionality of IAP, but also others factors including dietary regime and rearing condition system might have a role on it (Harpaz and Uni, 1999; Wu et al., 2009; Xiao et al., 2017). Studies that investigated IAP level changes correlated with fish ontogeny showed that although IAP is already present at larval hatching, also if at low levels; then it tends to increase with

development, reaching the first peak, and eventually a second, between 10/15 to 40 DAH (Ben Khemis et al., 2006; Ghasemi et al., 2020; Lallès, 2019; Zouiten et al., 2008). Despite this general trend, substantial differences in the temporal IAP pattern between species have been noted, as example both perch (*Perca fliviatilis* L.) and bay snook (*Petenia splendida*) displayed high IAP activity at hatching (Kuz'mina, 1996; Uscanga-Martínez et al., 2011). In the present study, alkaline phosphatase activity was not measured until 24 DAH, correlated to intestinal maturation. Differences in activity detection between treatments were only significant at 24 DAH, with higher values in CTRL larvae when compared to LOW larvae. Then, alkaline phosphatase increased in all treatments, suggesting an overall gain in larvae digestive capacity with development; nonetheless, the sharper increment was observed in larvae fed on LOW curcumin diet, suggesting a plausibile positive role of curcumin on intestinal maturation. Data collected by Lallès (2019) tend to support the hypothesis about the importance of feed intake as a major driver of IAP activity in fish. In fact, as observed in mammals (Goldberg et al., 2008; Lallès, 2014, 2010) and in fish, e.g. in Atlantic cod (*Gadus morhua*) (Lemieux et al., 1999), roach (*Rutiilus rutilus caspicus*) (Abolfathi et al., 2012) and blunt snout bream (*Megalobrama amblychepala)* (Xu et al., 2016) IAP activity is strongly and positive correlated with feed intake. In addition, as demonstrated by Engrola et al., (2007) in Senegalese sole larvae, the feeding transition at weaning is important in supporting GI development also at earlier stages of development. Finally, in this study, at 23 DAH seabream larvae fed on HIGH curcumin inclusion had significantly higher feed content in the gut than CTRL, although IAP activity was not different between treatments (at 24 DAH). This might indicate that feed intake did not significantly influence IAP activity. Nevertheless at 28 DAH, gut content of HIGH treatment was slightly lower than CTRL but three days after (i.e. 31 DAH) the level of IAP was substantially the same between treatments. So in conclusion, it is not clear if neither the presence of curcumin in the diet or feed intake might influence IAP activity in gilthead seabream larvae, but, as mentioned before, IAP activity followed an increasing trend in all the treatments, suggesting that curcumin was at least not negatively affecting the functionality of IAP. All this together, leave open the possibility for further studies to better investigate the role that curcumin might play as beneficial promoter of alkaline phosphatase functionality in the long-term, and therefore, on the general gut intestinal maturation.

In this study, feeding incidence was evaluated following the image analyses technique previously described by Romero-Romero and Yúfera, (2012) and optimized by Mata-Sotres et al., (2016). For each larva the level of gut fullness was determined measuring the pigmented area within the digestive cavity. Initially, the presence/absence of feed in the gut of each larvae was evaluated, indicating that along the experiment there were almost no differences in feeding incidence between treatments. In fact, differences were only observed at 8 DAH, where larvae fed on CTRL and LOW

diets presented higher feeding incidence than HIGH larvae. This confirmed that, overall, curcumin inclusion in the diet did not negatively affect neither the dietary palatability nor the larvae appetite. Moreover, along the complete experimental period, a wide variability in gut fullness was observed, especially for larvae fed on HIGH diet. Only CTRL treatment exhibited significant differences among samplings points with higher feeding incidence at 20 and 28 DAH, when compared to 8 and 12 DAH. Also, HIGH treatment showed differences close to the significance level, but globally, all the treatments increased the feeding incidence from 12 DAH-onwards, two days after the introduction of *Artemia* nauplii in the feeding plan. Secondly, gut fulness normalized for total length was taken under consideration. The results obtained from this analyse revealed no significant differences between treatments for almost all the experiment. The exceptions were at 12 and 23 DAH, where gut content was markedly higher in HIGH larvae. Despite this, both dry weight and total length measured at the nearest sampling point (10 and 24 DAH, respectively) were not significantly different between treatments, showing that probably larvae were not efficiently utilizing the higher level of ingested feed. Gut fullness showed a significant increment along the experimental period for all treatments. As seen previously for presence/absence of feed, the highest increment was here performed from 8 / 12 DAH onwards, coinciding to the inclusion of *Artemia* nauplii in the larval feeding plan. While weaning, did not affected negatively the larval feeding incidence, the overall survival and growth performance of larvae were lower than in others previously mentioned studies involving early weaning. This might probably indicate that the introduction of inert diet since mouth opening, combined with the reduction of live preys (both, in terms of quantity supplied and duration), is an extreme feeding plan that not fit well with the nutritional requirements of the young larvae or that may increase the stress level of the larvae rebounding negatively on performance indicators. As no differences were revealed between treatments, it was proved that curcumin did not have negative effects on survival and on key performance indicators; nevertheless, still remain to find out clear evidence about its positive role as intestinal maturation promoter from early stages of development.

In this study the effects of curcumin as a potent antioxidant agent were tested on gilthead fish larvae. In fact, while the effects of antioxidant agents such asα-tocopherol (vit E) and L-ascorbic acid (vit C) or taurine (Tau) have been already investigated in fish larvae (Chen et al., 2005, 2004; Matsunari et al., 2013; Pinto et al., 2018) and juveniles (Brotons Martinez et al., 2004; Kim et al., 2008, 2005, 2003; Takagi et al., 2008), curcumin was almost exclusively tested only in juveniles (Akdemir et al., 2017a; Jiang et al., 2016; Mahmoud et al., 2017a). Here the antioxidant status of the larvae was analysed through the measurement of differents biomarkers such as: total glutathione (TG), total antioxidant capacity (TAC), protein carbonylation (PC) and mitochondrial oxidative status (MOS), giving at the end a general picture of the antioxidant status of the

organism.Globally, the antioxidant molecules prevent and compensate the oxidative stress generated by the formation and the accumulation of harmfull oxidant agents: the so-called free radicals (Aksoy et al., 2013). Among these free radicals molecules, the reactive oxygens species (ROS) are one of the most studied due to their chemical unstability and their capacity to drive trough a oxidative stress condition (Young and Woodside, 2001). Oxidative stress is considered a physiological condition in which oxidant agents, mainly ROS, are continuosly produced within cells with an imbalanced rithm that overcome the production of antioxidant defenses scavenging the excessively produced ROS (Harwell, 2007; Imlay, 2003; Ott et al., 2007). If a condition of homeostasis is caractherized by the presence of antioxidant compounds that contrast and balance the harmful effects of oxidant molecules, in an oxidative stress condition the dangerous effects of these lasts molecules become even worse because the absence of the first (Kohen and Nyska, 2002). Thus, oxidant reactions can damage not only all sort of biomolecules within cells, such as proteins, lipids, carbohydrates and DNA but also the cell itself, culminating in cell death (Butterfield and Sultana, 2008; Levine and Stadtman, 2001).

Glutathione is a bioactive tripeptide present intracellularly in two forms: the reduced glutathione (GSH) and the oxidized glutathione (GSSG) (Ming et al., 2019). Glutathione redox cycle is one of the most important antioxidant defense mechanisms able to detoxify ROS, like hydrogen peroxide (Stephensen et al., 2002). GSH plays physiological functions in the synthesis of protein and DNA as well as acting as antioxidant agent reducing the rate of apoptosis in the different tissues, helping to maintain a proper cellular redox homeostasis, scavenging the free radicals molecules (Buzadžić et al., 2004; Deponte, 2013; Merad-Boudia et al., 1998; Ming et al., 2019; Saeij et al., 2003). Despite the role and the importance of GSH content in alleviate toxic effects of metals on animal at adult stages was analyzed and confirmed with tilapia (Atli and Canli, 2008), rats (Chattopadhyay and Ghosh, 2010) and grass carp juveniles (Ming et al., 2019), there are not studies investigating how to enhance the antioxidant capacity of fish larvae, specifically through the induction of GSH rises. In this study no differences were observed between treatments in TG content; this might mean that curcumin did not played any significant role in the promotion of glutathione production, and therefore glutathione redox cycle. In fact, if in terms of average content during the period of the experiment TG levels were sligthly higher in larvae fed on LOW curcumin, but with a higher average deviation, by contrast, larvae fed on commercial diet presented both, a tend to lower TG content and lower deviation.

The antioxidant capacity of an organism, or sample, is measured as the amount of antioxidant molecules, considered as a whole. The fact that the antioxidant molecules are considered as a whole instead of being separated, depends on two factors: the first implies the practical difficulty of

measuring these molecules individually, while the second involves their additive property at the base of their function (Erel, 2004). From this last point derives that the antioxidant capacity is also called total antioxidant capacity (TAC) (Erel, 2004; Rice-Evans and Miller, 1994). As previously reported, the antioxidant molecules prevent, or inhibit, the harmfull oxidant reactions caused by the presence of some unstable molecules, such ROS. The capacity of an organism to not incur in oxidative stress condition will be higher the greater the availability of antioxidant molecules (Barbosa et al., 2020). In this study, TAC of larvae from different treatments followed the same pattern, slightly decreased from 10 DAH to 24 DAH and then slightly increased until 31 DAH, although no differences were noticed between treatments along the experiment. Results suggest that curcumin did not significantly stimulate the larval total antioxidant capacity, neither positively nor negatively. This fact might indicate a positive role of curcumin as antioxidant promoter also in the larval stage; being in line with that described in the literature for juvenile and adult fish like in Giri et al. (2019) for common carp, in Ge et al. (2015) for Wuchang bream (*Megalobrama amblycephala*) and in Mahmoud et al. (2017b) for tilapia.

The posttranslational modifications (PTMs) are one of the most harmful cellular effects caused by ROS. These modifications affect in particularly biomolecules like proteins, due to their large abundance in cells (Bollineni et al., 2014). Protein carbonylation (PC) is among the PTMs that affect proteins, and is widely recognized as marker of oxidative stress under pathological conditions (Dalle-Donne et al., 2003). PC consist in an irreversible oxidation of the amino acid chain yielding chemically reactive carbonyl groups, such as aldehydes, ketones or lactams (Fedorova et al., 2014). These compounds, accumulated in the cells, cause the breakages of the normal functionality of the organism driving to the appearance of diseases (Stadtman, 2006). In the present work it was expected a reduction of PC in the larvae fed on dietary curcumin, as indicative of a higher antioxidant status. At 10 DAH, PC tended to be lower in HIGH treatment, being close to the significant level, suggesting that curcumin was probably reducing the carbonylation rate of proteins. In addition, among treatment with curcumin supplementation, HIGH diet seemed to be more efficient than LOW diet, which showed the highest value measured at this stage. Along the experiment, PC levels of HIGH larvae showed an oscillatory pattern, increasing at 24 DAH to later decline at 31 DAH. Only HIGH larvae showed a decreasing PC pattern between 24 and 31 DAH, suggesting a positive curcumin effect on PC levels. Further studies need to be conducted trying to enlarge the rearing period and trying to test different curcumin concentrations to find the one that better optimize the reduction of both, PC levels and heterogeneity of the results.

Studies on cell cultures, both of invertebrates and mammals, support the idea that ROS, especially the ones produced in the mitochondria, play an essential role in aging and scenecence processes

(Balaban et al., 2005; Barja, 2014, 2004). In fact, with age the oxidative stress within mitochondria increases and the accumulation of oxidant products might damage not only the macromolecules, such as lipids, proteins and DNA, but also negatively affect the mitochondrial function (Paradies et al., 2011; Shigenaga et al., 1994; Sohal et al., 2002). In the present study, curcumin was tested also as promoter of mitochondrial antioxidant pathways, with the objective of reducing as much as possible the oxidative stress condition of these organelles. The mitochondrial oxidative status followed an increasing ontogenetic trend in all treatments, as previously reported for zebrafish (*Danio rerio*) in Almaida-Pagán et al. (2014). Nevertheless, only in larvae fed on the commercial diet this increment was significant. Thus, curcumin might have played a significative role in the mitigation of the stress occurred at mitochondrial level. This is evident, in particular, considering the last period of the trials, in which MOS pattern was even negative in LOW larvae, while it was increasing in the other treatments. At the end, results suggest that diet with LOW curcumin concentration was generally acting positively on MOS.

In general terms, the dietary treatments tested in the present study started to shed some light on a possible antioxidant supplementation on larval diets to promote overall robustness. Some antioxidant status biomarkers showed interesting patterns that might be further investigated to extend the knowledge of curcumin effects on fish larvae.

5. Conclusion

In this trial, metabolic programming and nutritional concepts have been combined in order to promote larvae fish robustness. A general trend to higher key performance indicators values was detected in larvae fed on LOW diet, revealing a plausible positive effect of curcumin supplementation that could have higher impacts in the long-term.

Digestive capacity was promoted through the dietary curcumin supplementation. Trypsin higher activity was observed in larvae fed HIGH diet, concomitantly with a higher activity of chymotrypsin in larvae from fed supplemented diets, suggesting that dietary curcumin might promote larval proteolytic capacity. Antioxidant biomarkers did not reveal a clear effect of curcumin as antioxidant promoter. However, some trends might indicate a positive effect of curcumin, as in the case of mithocondrial oxidative status.

In general, further early programming studies need to be conducted in order to investigate deeper the role of curcumin as growth promoter and antioxidant agent. Moreover, the results of the present study leave open, and possibly encourage, further research on early weaning feeding regimes and dietary curcumin supplementation.

6. References

Abolfathi, M., Hajimoradloo, A., Ghorbani, R., Zamani, A., 2012. Effect of starvation and refeeding on digestive enzyme activities in juvenile roach, Rutilus rutilus caspicus. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 161, 166–173. https://doi.org/10.1016/j.cbpa.2011.10.020

Abrahams, S., Haylett, W.L., Johnson, G., Carr, J.A., Bardien, S., 2019. Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: A review. Neuroscience. https://doi.org/10.1016/j.neuroscience.2019.02.020

Abramoff, M.D.., Magalhães, P.J.., Ram, S.J., 2006. Image Processing with ImageJ, in: Optical Imaging Techniques in Cell Biology. CRC Press, pp. 249–258. https://doi.org/10.1201/9781420005615.ax4

Aggarwal, B.B., Sung, B., 2009. Pharmacological basis for the role of curcumin in chronic diseases: an age-old spice with modern targets. Trends in Pharmacological Sciences 30, 85–94. https://doi.org/10.1016/j.tips.2008.11.002

Ahmad, I., Hamid, T., Fatima, M., Chand, H.S., Jain, S.K., Athar, M., Raisuddin, S., 2000. Induction of hepatic antioxidants in freshwater catfish (Channa punctatus Bloch) is a biomarker of paper mill effluent exposure. Biochimica et Biophysica Acta - General Subjects. https://doi.org/10.1016/S0304-4165(00)00098-2

Akdemir, F., Orhan, C., Tuzcu, M., Sahin, N., Juturu, V., Sahin, K., 2017a. The efficacy of dietary curcumin on growth performance, lipid peroxidation and hepatic transcription factors in rainbow trout *Oncorhynchus Mykiss* (Walbaum) reared under different stocking densities. Aquaculture Research 48, 4012–4021. https://doi.org/10.1111/are.13223

Akdemir, F., Orhan, C., Tuzcu, M., Sahin, N., Juturu, V., Sahin, K., 2017b. The efficacy of dietary curcumin on growth performance, lipid peroxidation and hepatic transcription factors in rainbow trout Oncorhynchus Mykiss (Walbaum) reared under different stocking densities. Aquaculture Research 48, 4012–4021. https://doi.org/10.1111/are.13223

Aksoy, L., Kolay, E., Aĝilönü, Y., Aslan, Z., Kargioĝlu, M., 2013. Free radical scavenging activity, total phenolic content, total antioxidant status, and total oxidant status of endemic Thermopsis turcica. Saudi Journal of Biological Sciences. https://doi.org/10.1016/j.sjbs.2013.02.003

Al-Sagheer, A.A., Mahmoud, H.K., Reda, F.M., Mahgoub, S.A., Ayyat, M.S., 2018. Supplementation of diets for Oreochromis niloticus with essential oil extracts from lemongrass (Cymbopogon citratus) and geranium (Pelargonium graveolens) and effects on growth, intestinal microbiota, antioxidant and immune activities. Aquaculture Nutrition. https://doi.org/10.1111/anu.12637

Almaida-Pagán, P.F., Lucas-Sánchez, A., Tocher, D.R., 2014. Changes in mitochondrial membrane composition and oxidative status during rapid growth, maturation and aging in zebrafish, Danio rerio. Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids. https://doi.org/10.1016/j.bbalip.2014.04.004

Anderson, O.S., Sant, K.E., Dolinoy, D.C., 2012. Nutrition and epigenetics: an interplay of dietary methyl donors, one-carbon metabolism and DNA methylation. The Journal of Nutritional Biochemistry 23, 853–859. https://doi.org/10.1016/j.jnutbio.2012.03.003

Arikan, M.S., Aral, Y., 2019. Economic analysis of aquaculture enterprises and determination of factors affecting sustainability of the sector in Turkey. Ankara Universitesi Veteriner Fakultesi Dergisi. https://doi.org/10.1501/Vetfak_0000002888

Asche, F., Bellemare, M.F., Roheim, C., Smith, M.D., Tveteras, S., 2015. Fair Enough? Food Security and the International Trade of Seafood. World Development. https://doi.org/10.1016/j.worlddev.2014.10.013

Atli, G., Canli, M., 2008. Responses of metallothionein and reduced glutathione in a freshwater fish Oreochromis niloticus following metal exposures. Environmental Toxicology and Pharmacology. https://doi.org/10.1016/j.etap.2007.08.007

Avanço, G.B., Ferreira, F.D., Bomfim, N.S., Santos, P.A. de S.R. dos, Peralta, R.M., Brugnari, T., Mallmann, C.A., Abreu Filho, B.A. de, Mikcha, J.M.G., Machinski Jr., M., 2017. *Curcuma longa* L. essential oil composition, antioxidant effect, and effect on Fusarium verticillioides and fumonisin production. Food Control 73, 806–813. https://doi.org/10.1016/j.foodcont.2016.09.032

Baker, M.A., Cerniglia, G.J., Zaman, A., 1990. Microtiter plate assay for the measurement of glutathione and glutathione disulfide in large numbers of biological samples. Analytical Biochemistry. https://doi.org/10.1016/0003-2697(90)90208-Q

Balaban, R.S., Nemoto, S., Finkel, T., 2005. Mitochondria, oxidants, and aging. Cell. https://doi.org/10.1016/j.cell.2005.02.001

Baldissera, M.D., Souza, C.F., Zeppenfeld, C.C., Descovi, S., Machado, V.S., Santos, R.C.V., Baldisserotto, B., 2018. Efficacy of dietary curcumin supplementation as bactericidal for silver catfish against Streptococcus agalactiae. Microbial Pathogenesis 116, 237–240. https://doi.org/10.1016/j.micpath.2018.01.044

Barazi Yeroulanos, L., 2010. Synthesis of Mediterranean Marine Finfish Aquaculture - a Marketing and Promotion Strategy, Studies and Reviews - General Fisheries Commission for the Mediterranean 2010.

Barbosa, M.L., de Meneses, A.A.P.M., de Aguiar, R.P.S., de Castro e Sousa, J.M., de Carvalho Melo Cavalcante, A.A., Maluf, S.W., 2020. Oxidative stress, antioxidant defense and

depressive disorders: A systematic review of biochemical and molecular markers. Neurology Psychiatry and Brain Research. https://doi.org/10.1016/j.npbr.2020.02.006

Barja, G., 2014. The mitochondrial free radical theory of aging, in: Progress in Molecular Biology and Translational Science. https://doi.org/10.1016/B978-0-12-394625-6.00001-5

Barja, G., 2004. Free radicals and aging. Trends in Neurosciences. https://doi.org/10.1016/j.tins.2004.07.005

Basto, A., Matos, E., Valente, L.M.P., 2020. Nutritional value of different insect larvae meals as protein sources for European sea bass (Dicentrarchus labrax) juveniles. Aquaculture. https://doi.org/10.1016/j.aquaculture.2020.735085

Bauchot, M.-L. and J.-C.H., 1990. Check-list of the fishes of the eastern tropical Atlantic (Clofeta). In J.C. Quero, J.C. Hureau, C. Karrer, A. Post and L. Saldanha (eds.) JNICT, Lis, 790-812.

Belghit, I., Liland, N.S., Gjesdal, P., Biancarosa, I., Menchetti, E., Li, Y., Waagbø, R., Krogdahl, Å., Lock, E.J., 2019. Black soldier fly larvae meal can replace fish meal in diets of sea-water phase Atlantic salmon (Salmo salar). Aquaculture. https://doi.org/10.1016/j.aquaculture.2018.12.032

Bellio, P., Brisdelli, F., Perilli, M., Sabatini, A., Bottoni, C., Segatore, B., Setacci, D., Amicosante, G., Celenza, G., 2014. Curcumin inhibits the SOS response induced by levofloxacin in *Escherichia coli*. Phytomedicine 21, 430–434. https://doi.org/10.1016/j.phymed.2013.10.011

Belton, B., Bush, S.R., Little, D.C., 2018. Not just for the wealthy: Rethinking farmed fish consumption in the Global South. Global Food Security. https://doi.org/10.1016/j.gfs.2017.10.005

Ben Khemis, I., Zouiten, D., Besbes, R., Kamoun, F., 2006. Larval rearing and weaning of thick lipped grey mullet (Chelon labrosus) in mesocosm with semi-extensive technology. Aquaculture. https://doi.org/10.1016/j.aquaculture.2006.05.007

Béné, C., Barange, M., Subasinghe, R., Pinstrup-Andersen, P., Merino, G., Hemre, G.I., Williams, M., 2015. Feeding 9 billion by 2050 – Putting fish back on the menu. Food Security. https://doi.org/10.1007/s12571-015-0427-z

Beveridge, M.C.M., Thilsted, S.H., Phillips, M.J., Metian, M., Troell, M., Hall, S.J., 2013. Meeting the food and nutrition needs of the poor: the role of fish and the opportunities and challenges emerging from the rise of aquaculture a. Journal of Fish Biology 83(4), 1067–1084. https://doi.org/10.1111/jfb.12187

Bilski, J., Mazur-Bialy, A., Wojcik, D., Zahradnik-Bilska, J., Brzozowski, B., Magierowski, M., Mach, T., Magierowska, K., Brzozowski, T., 2017. The Role of Intestinal Alkaline Phosphatase in Inflammatory Disorders of Gastrointestinal Tract. Mediators of Inflammation. https://doi.org/10.1155/2017/9074601

Bolasina, S., Pérez, A., Yamashita, Y., 2006. Digestive enzymes activity during ontogenetic development and effect of starvation in Japanese flounder, Paralichthys olivaceus. Aquaculture 252, 503–515. https://doi.org/10.1016/j.aquaculture.2005.07.015

Bollineni, R.C., Hoffmann, R., Fedorova, M., 2014. Proteome-wide profiling of carbonylated proteins and carbonylation sites in HeLa cells under mild oxidative stress conditions. Free Radical Biology and Medicine. https://doi.org/10.1016/j.freeradbiomed.2013.11.030

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry. https://doi.org/10.1016/0003-2697(76)90527-3

Brotons Martinez, J., Chatzifotis, S., Divanach, P., Takeuchi, T., 2004. Effect of dietary taurine supplementation on growth performance and feed selection of sea bass Dicentrarchus labrax fry fed with demand-feeders. Fisheries Science. https://doi.org/10.1111/j.1444-2906.2003.00773.x

Buhrmann, C., Yazdi, M., Bashiri Dezfouli, A., Samani Sahraneshin, F., Ebrahimi, S.M., Hamidollah Ghaffari, S., Yaghmaie, M., Barin, A., Shakibaei, M., Shayan, P., 2020. Significant decrease in the viability and tumor stem cell marker expression in tumor cell lines treated with curcumin. Journal of Herbal Medicine 100339. https://doi.org/10.1016/j.hermed.2020.100339

Burdge, G.C., Lillycrop, K.A., 2010. Nutrition, Epigenetics, and Developmental Plasticity: Implications for Understanding Human Disease. Annual Review of Nutrition 30, 315–339. https://doi.org/10.1146/annurev.nutr.012809.104751

Butterfield, D.A., Sultana, R., 2008. Redox proteomics: Understanding oxidative stress in the progression of age-related neurodegenerative disorders. Expert Review of Proteomics. https://doi.org/10.1586/14789450.5.2.157

Buzadžić, B., Korać, A., Petrović, V., Korać, B., 2004. Glutathion content, rate of apoptosis, and brown adipose tissue mass in rats exposed to different ambient temperatures, in: Journal of Thermal Biology. https://doi.org/10.1016/j.jtherbio.2004.08.082

Cahu, C., Zambonino Infante, J., 2001. Substitution of live food by formulated diets in marine fish larvae, in: Aquaculture. pp. 161–180. https://doi.org/10.1016/S0044-8486(01)00699-8

Cahu, C.L., Infante, J.L.Z., 1995. Effect of the molecular form of dietary nitrogen supply in sea bass larvae: Response of pancreatic enzymes and intestinal peptidases. Fish Physiology and Biochemistry. https://doi.org/10.1007/BF00004311

Cahu, C.L., Infante, J.L.Z., 1994. Early weaning of sea bass (Dicentrarchus labrax) larvae with a compound diet: Effect on digestive enzymes. Comparative Biochemistry and Physiology -- Part A: Physiology. https://doi.org/10.1016/0300-9629(94)90123-6

Cahu, C.L., Infante, J.L.Z., Barbosa, V., 2003. Effect of dietary phospholipid level and phospholipid:neutral lipid value on the development of sea bass (Dicentrarchus labrax) larvae fed a compound diet . British Journal of Nutrition. https://doi.org/10.1079/bjn2003880

Campos, I., Pinheiro Valente, L.M., Matos, E., Marques, P., Freire, F., 2020. Life-cycle assessment of animal feed ingredients: Poultry fat, poultry by-product meal and hydrolyzed feather meal. Journal of Cleaner Production. https://doi.org/10.1016/j.jclepro.2019.119845

Canada, P., Engrola, S., Conceição, L.E.C., Valente, L.M.P., 2019. Improving growth potential in Senegalese sole (*Solea senegalensis*) through dietary protein. Aquaculture 498, 90–99. https://doi.org/10.1016/j.aquaculture.2018.08.044

Cara, B., Moyano, F.J., Zambonino, J.L., Fauvel, C., 2007. Trypsin and chymotrypsin as indicators of nutritional status of post-weaned sea bass larvae. Journal of Fish Biology 70, 1798–1808. https://doi.org/10.1111/j.1095-8649.2007.01457.x

Cara, J.B., Moyano, F.J., Cardenas, S., Fernandez-Diaz, C., Yufera, M., 2003. Assessment of digestive enzyme activities during larval development of white bream. Journal of Fish Biology 63, 48–58. https://doi.org/10.1046/j.1095-8649.2003.00120.x

Chattopadhyay, S., Ghosh, D., 2010. Role of dietary GSH in the amelioration of sodium arsenite-induced ovarian and uterine disorders. Reproductive Toxicology. https://doi.org/10.1016/j.reprotox.2010.05.002

Chen, B.N., Qin, J.G., Kumar, M.S., Hutchinson, W.G., Clarke, S.M., 2006. Ontogenetic development of digestive enzymes in yellowtail kingfish Seriola lalandi larvae. Aquaculture 260, 264–271. https://doi.org/10.1016/j.aquaculture.2006.06.021

Chen, J.N., Takeuchi, T., Takahashi, T., Tomoda, T., Koiso, M., Kuwada, H., 2005. Effect of rotifers enriched with taurine on growth in larvae of Japanese flounder Paralichthys olivaceus. Nippon Suisan Gakkaishi (Japanese Edition). https://doi.org/10.2331/suisan.71.342

Chen, J.N., Takeuchi, T., Takahashi, T., Tomoda, T., Koiso, M., Kuwada, H., 2004. Effect of rotifers enriched with taurine on growth and survival activity of red sea bream Pagrus major larvae. Nippon Suisan Gakkaishi (Japanese Edition). https://doi.org/10.2331/suisan.70.542

Clarkson, M., Migaud, H., Metochis, C., Vera, L.M., Leeming, D., Tocher, D.R., Taylor, J.F., 2017. Early nutritional intervention can improve utilisation of vegetable-based diets in diploid and triploid Atlantic salmon (Salmo salar L.). British Journal of Nutrition 118, 17–29. https://doi.org/10.1017/S0007114517001842

Conceição, L.E.C., Dersjant-Li, Y., Verreth, J.A.J., 1998. Cost of growth in larval and juvenile African catfish (Clarias gariepinus) in relation to growth rate, food intake and oxygen consumption, in: Aquaculture. https://doi.org/10.1016/S0044-8486(97)00260-3

Costa, J.., 2012. Sea Bream (Sparus Aurata) Intensive Larval Rearing: Effects of Microalgae and

Phages Addition on the Performance of the Larvae (MSc thesis).

Cui, H., Liu, B., Ge, X., Xie, J., Xu, P., Miao, L., Sun, S., Liao, Y., Chen, R., Ren, M., Zhou, Q., Pan, L., 2013. Effects of dietary curcumin on growth performance, biochemical parameters, HSP70 gene expression and resistance to Streptococcus iniae of juvenile Gift Tilapia, *Oreochromis niloticus*. The Israeli Journal of Aquaculture Bamidgeh, 11 pages.

da Silva, A.I., Braz, G.R.F., Pedroza, A.A., Nascimento, L., Freitas, C.M., Ferreira, D.J.S., Manhães de Castro, R., Lagranha, C.J., 2015. Fluoxetine induces lean phenotype in rat by increasing the brown/white adipose tissue ratio and UCP1 expression. Journal of Bioenergetics and Biomembranes. https://doi.org/10.1007/s10863-015-9617-9

Dalle-Donne, I., Giustarini, D., Rossi, R., Colombo, R., Milzani, A., 2003. Reversible S-glutathionylation of Cys374 regulates actin filament formation by inducing structural changes in the actin molecule. Free Radical Biology and Medicine. https://doi.org/10.1016/S0891-5849(02)01182-6

Daprà, F., Geurden, I., Corraze, G., Bazin, D., Zambonino-Infante, J.L., Fontagné-Dicharry, S., 2011. Physiological and molecular responses to dietary phospholipids vary between fry and early juvenile stages of rainbow trout (Oncorhynchus mykiss). Aquaculture. https://doi.org/10.1016/j.aquaculture.2011.07.016

Deponte, M., 2013. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. Biochimica et Biophysica Acta - General Subjects. https://doi.org/10.1016/j.bbagen.2012.09.018

Diaz, J.P., Mani-Ponset, L., Blasco, C., Connes, R., 2002. Cytological detection of the main phases of lipid metabolism during early post-embryonic development in three teleost species: Dicentrarchus labrax, Sparus aurata and Stizostedion lucioperca. Aquatic Living Resources. https://doi.org/10.1016/S0990-7440(02)01169-5

Elbal, M.T., García Hernández, M.P., Lozano, M.T., Agulleiro, B., 2004. Development of the digestive tract of gilthead sea bream (Sparus aurata L.). Light and electron microscopic studies. Aquaculture. https://doi.org/10.1016/j.aquaculture.2003.11.028

Engrola, S., Conceição, L.E.C., Dias, L., Pereira, R., Ribeiro, L., Dinis, M.T., 2007. Improving weaning strategies for Senegalese sole: Effects of body weight and digestive capacity. Aquaculture Research. https://doi.org/10.1111/j.1365-2109.2007.01701.x

Engrola, S., Dinis, M.T., Conceicao, L.E.C., 2010. Senegalese sole larvae growth and protein utilization is depressed when co-fed high levels of inert diet and Artemia since first feeding. Aquaculture Nutrition 16, 457–465. https://doi.org/10.1111/j.1365-2095.2009.00682.x

Engrola, S., Figueira, L., Conceição, L.E.C., Gavaia, P.J., Ribeiro, L., Dinis, M.T., 2009. Co-feeding in Senegalese sole larvae with inert diet from mouth opening promotes growth at

weaning. Aquaculture 288, 264–272. https://doi.org/10.1016/j.aquaculture.2008.12.010

Erel, O., 2004. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clinical Biochemistry. https://doi.org/10.1016/j.clinbiochem.2003.11.015

Fang, L., Liang, X.-F., Zhou, Y., Guo, X.-Z., He, Y., Yi, T.-L., Liu, L.-W., Yuan, X.-C., Tao, Y.-X., 2014. Programming effects of high-carbohydrate feeding of larvae on adult glucose metabolism in zebrafish, *Danio rerio*. British Journal of Nutrition 111, 808–818. https://doi.org/10.1017/S0007114513003243

FAO, 2020a. GLOBEFISH Highlights January 2020 ISSUE, with Jan. – Sep. 2019 Statistics. FAO. https://doi.org/10.4060/ca7968en

FAO, 2020b. The State of World Fisheries and Aquaculture 2020. FAO. https://doi.org/10.4060/ca9229en

FAO, 2019. The State of the World's Biodiversity for Food and Agriculture: FAO Commission on Genetic Resources for Food and Agricultre Assessments, Fao. https://doi.org/978-92-5-131270-4

FAO, 2018. FishStatJ [WWW Document]. URL http://www.fao.org/fishery/statistics/software/fishstatj/en,

Farhangi, B., Alizadeh, A.M., Khodayari, H., Khodayari, S., Dehghan, M.J., Khori, V., Heidarzadeh, A., Khaniki, M., Sadeghiezadeh, M., Najafi, F., 2015. Protective effects of dendrosomal curcumin on an animal metastatic breast tumor. European Journal of Pharmacology 758, 188–196. https://doi.org/10.1016/j.ejphar.2015.03.076

FEAP, 2014. Annual Report 2014. Federation of European Aquaculture Producers, Belgium.

Fedorova, M., Bollineni, R.C., Hoffmann, R., 2014. Protein carbonylation as a major hallmark of oxidative damage. Mass Spectrom Rev. https://doi.org/10.1002/mas.21381 T4 - update of analytical strategies PM - 23832618

Fernley, H., Walker, P., 1965. Kinetic behaviour of calf-intestinal alkaline phosphatase with 4-methylumbelliferyl phosphate. Biochemical Journal. https://doi.org/10.1042/bj0970095

Fezzardi, D., Massa, F., Avila-Zaragoza, P., Rad, F., Yucel-Gier, G., Deniz, H., Salem, M.H.A., Hamza, H.A.B.S., 2013. Indicators for sustainable aquaculture in Mediterranean and Black Sea countries. Studies and Reviews. General Fisheries Commission for the Mediterranean. No 93. Rom, 60 pp. https://doi.org/10.13140/2.1.3789.5366

Føre, M., Frank, K., Norton, T., Svendsen, E., Alfredsen, J.A., Dempster, T., Eguiraun, H., Watson, W., Stahl, A., Sunde, L.M., Schellewald, C., Skøien, K.R., Alver, M.O., Berckmans, D., 2018. Precision fish farming: A new framework to improve production in aquaculture. Biosystems Engineering 173, 176–193. https://doi.org/10.1016/j.biosystemseng.2017.10.014

Garcia-Ruiz, C., Colell, A., Morales, A., Kaplowitz, N., Fernandez-Checa, J.C., 1995. Role of oxidative stress generated from the mitochondrial electron transport chain and mitochondrial glutathione status in loss of mitochondrial function and activation of transcription factor nuclear factor-κB: Studies with isolated mitochondria and rat . Molecular Pharmacology.

Gawlicka, A., Parent, B., Horn, M.H., Ross, N., Opstad, I., Torrissen, O.J., 2000. Activity of digestive enzymes in yolk-sac larvae of Atlantic halibut (Hippoglossus hippoglossus): Indication of readiness for first feeding. Aquaculture. https://doi.org/10.1016/S0044-8486(99)00322-1

Ge, X.P., Liu, B., Xia, S.L., Xie, J., Miao, L.H., Ren, M.C., Zhou, Q.L., Zhang, W.X., Jiang, X.J., Chen, R.L., Pan, L.K., 2015. Effects of supplemented dietary curcumin on growth and non-Specific immune responses in juvenile wuchang bream (*megalobrama amblycephala*). Israeli Journal of Aquaculture - Bamidgeh.

Geurden, I., Jutfelt, F., Olsen, R.E., Sundell, K.S., 2009. A vegetable oil feeding history affects digestibility and intestinal fatty acid uptake in juvenile rainbow trout Oncorhynchus mykiss. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology. https://doi.org/10.1016/j.cbpa.2008.12.016

Ghasemi, N., Imani, A., Noori, F., Shahrooz, R., 2020. Ontogeny of digestive tract of Stellate sturgeon (Acipenser stellatus) from hatching to juvenile stage: Digestive enzymes activity, stomach and proximal intestine. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.734751

Giri, S.S., Sukumaran, V., Park, S.C., 2019. Effects of bioactive substance from turmeric on growth, skin mucosal immunity and antioxidant factors in common carp, *Cyprinus carpio*. Fish & Shellfish Immunology 92, 612–620. https://doi.org/10.1016/j.fsi.2019.06.053

Gisbert, E., Conklin, D.B., Piedrahita, R.H., 2004. Effects of delayed first feeding on the nutritional condition and mortality of California halibut larvae. Journal of Fish Biology. https://doi.org/10.1111/j.1095-8649.2004.00289.x

Gisbert, E., Giménez, G., Fernández, I., Kotzamanis, Y., Estévez, A., 2009. Development of digestive enzymes in common dentex Dentex dentex during early ontogeny. Aquaculture. https://doi.org/10.1016/j.aquaculture.2008.10.039

GLOBEFISH, 2017. Proactive Action Pays off for Seabass and Seabream Sector. Seabass and Seabream Market [WWW Document]. Reports.

Goldberg, R.F., Austen, W.G., Zhang, X., Munene, G., Mostafa, G., Biswas, S., McCormack, M., Eberlin, K.R., Nguyen, J.T., Tatlidede, H.S., Warren, H.S., Narisawa, S., Millán, J.L., Hodin, R.A., 2008. Intestinal alkaline phosphatase is a gut mucosal defense factor maintained by enteral nutrition. Proceedings of the National Academy of Sciences of the United States of

America. https://doi.org/10.1073/pnas.0712140105

Gong, G., Xue, M., Wang, J., Wu, X., Zheng, Y., Han, F., Liang, X., Su, X., 2015. The regulation of gluconeogenesis in the Siberian sturgeon (*Acipenser baerii*) affected later in life by a short-term high-glucose programming during early life. Aquaculture 436, 127–136. https://doi.org/10.1016/j.aquaculture.2014.10.044

Greathead, H., 2003. Plants and plant extracts for improving animal productivity. Proceedings of the Nutrition Society 62, 279–290. https://doi.org/10.1079/PNS2002197

Grigorakis, K., Rigos, G., 2011. Aquaculture effects on environmental and public welfare – The case of Mediterranean mariculture. Chemosphere 85, 899–919. https://doi.org/10.1016/j.chemosphere.2011.07.015

Guil-Guerrero, J., Ramos, L., Zúñiga Paredes, J., Carlosama-Yépez, M., Moreno, C., Ruales, P., 2017. Effects of turmeric rhizome powder and curcumin in poultry production. A review. Journal of Animal and Feed Sciences 26, 293–302. https://doi.org/10.22358/jafs/78511/2017

Guillen, J., Natale, F., Fernández Polanco, J.M., 2015. Estimating the economic performance of the EU aquaculture sector. Aquaculture International 23, 1387–1400. https://doi.org/10.1007/s10499-015-9891-x

Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L.E.C., Izquierdo, M., 2013. Fish larval nutrition and feed formulation: Knowledge gaps and bottlenecks for advances in larval rearing. Reviews in Aquaculture. https://doi.org/10.1111/j.1753-5131.2012.01086.x

Harpaz, S., Uni, Z., 1999. Activity of intestinal mucosal brush border membrane enzymes in relation to the feeding habits of three aquaculture fish species. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology. https://doi.org/10.1016/S1095-6433(99)00106-3

Harwell, B., 2007. Biochemistry of oxidative stress, in: Biochemical Society Transactions. https://doi.org/10.1042/BST0351147

Hicks, C.C., Cohen, P.J., Graham, N.A.J., Nash, K.L., Allison, E.H., D'Lima, C., Mills, D.J., Roscher, M., Thilsted, S.H., Thorne-Lyman, A.L., MacNeil, M.A., 2019. Harnessing global fisheries to tackle micronutrient deficiencies. Nature. https://doi.org/10.1038/s41586-019-1592-6

High Level Panel of Experts on World Food Security, 2014. Sustainable fisheries and aquaculture for food security and nutrition, FAO.

Hosseini-Vashan, S.J., Safdari-Rostamabad, M., Piray, A.H., Sarir, H., 2020. The growth performance, plasma biochemistry indices, immune system, antioxidant status, and intestinal morphology of heat-stressed broiler chickens fed grape (Vitis vinifera) pomace. Animal Feed Science and Technology. https://doi.org/10.1016/j.anifeedsci.2019.114343

Hua, K., Bureau, D.P., 2012. Exploring the possibility of quantifying the effects of plant protein ingredients in fish feeds using meta-analysis and nutritional model simulation-based approaches. Aquaculture. https://doi.org/10.1016/j.aquaculture.2012.05.003

Ibrahim, R.E., El-Houseiny, W., Behairy, A., Abo-Elmaaty, A., Al-Sagheer, A.A., 2019. The palliative role of Eruca sativa leaves dietary supplementation against oxidative stress, immunosuppression, and growth retardation in temperature-stressed Oreochromis niloticus. Journal of Thermal Biology. https://doi.org/10.1016/j.jtherbio.2019.05.026

Ilsley, S.E., Miller, H.M., Kamel, C., 2005. Effects of dietary quillaja saponin and curcumin on the performance and immune status of weaned piglets1. Journal of Animal Science 83, 82–88. https://doi.org/10.2527/2005.83182x

Imlay, J.A., 2003. Pathways of Oxidative Damage. Annual Review of Microbiology. https://doi.org/10.1146/annurev.micro.57.030502.090938

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernández-Cruz, C.M., 2000a. Recent advances in lipid nutrition in fish larvae. Fish Physiology and Biochemistry. https://doi.org/10.1023/A:1007810506259

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernández-Cruz, C.M., 2000b. Recent advances in lipid nutrition in fish larvae. Fish Physiology and Biochemistry. https://doi.org/10.1023/A:1007810506259

Izquierdo, M.S., Turkmen, S., Montero, D., Zamorano, M.J., Afonso, J.M., Karalazos, V., Fernández-Palacios, H., 2015. Nutritional programming through broodstock diets to improve utilization of very low fishmeal and fish oil diets in gilthead sea bream. Aquaculture 449, 18–26. https://doi.org/10.1016/j.aquaculture.2015.03.032

Jiang, J., Wu, X.Y., Zhou, X.Q., Feng, L., Liu, Y., Jiang, W.D., Wu, P., Zhao, Y., 2016. Effects of dietary curcumin supplementation on growth performance, intestinal digestive enzyme activities and antioxidant capacity of crucian carp *Carassius auratus*. Aquaculture. https://doi.org/10.1016/j.aquaculture.2016.05.040

Jobling, M., 2016. Fish nutrition research: past, present and future. Aquaculture International 24, 767–786. https://doi.org/10.1007/s10499-014-9875-2

Jobling, M., 2011. M. A. Pavlidis and C. C. Mylonas (eds): Sparidae: biology and aquaculture of gilthead sea bream and other species. Aquaculture International 19, 809–810. https://doi.org/10.1007/s10499-011-9455-7

Kemski, M., Wick, M., Dabrowski, K., 2018. Nutritional programming effects on growth and reproduction of broodstock and embryonic development of progeny in yellow perch (Perca flavescens) fed soybean meal-based diets. Aquaculture 497, 452–461. https://doi.org/10.1016/j.aquaculture.2018.07.001

Kim, S.K., Matsunari, H., Nomura, K., Tanaka, H., Yokoyama, M., Murata, Y., Ishihara, K., Takeuchi, T., 2008. Effect of dietary taurine and lipid contents on conjugated bile acid composition and growth performance of juvenile Japanese flounder Paralichthys olivaceus. Fisheries Science. https://doi.org/10.1111/j.1444-2906.2008.01602.x

Kim, S.K., Takeuchi, T., Akimoto, A., Furuita, H., Yamamoto, T., Yokoyama, M., Murata, Y., 2005. Effect of taurine supplemented practical diet on growth performance and taurine contents in whole body and tissues of juvenile Japanese flounder Paralichthys olivaceus. Fisheries Science. https://doi.org/10.1111/j.1444-2906.2005.01008.x

Kim, S.K., Takeuchi, T., Yokoyama, M., Murata, Y., 2003. Effect of dietary supplementation with taurine, β-alanine and GABA on the growth of juvenile and fingerling Japanese flounder Paralichthys olivaceus. Fisheries Science. https://doi.org/10.1046/j.1444-2906.2003.00614.x

Kohen, R., Nyska, A., 2002. Oxidation of biological systems: Oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicologic Pathology. https://doi.org/10.1080/01926230290166724

Kok, B., Malcorps, W., Tlusty, M.F., Eltholth, M.M., Auchterlonie, N.A., Little, D.C., Harmsen, R., Newton, R.W., Davies, S.J., 2020. Fish as feed: Using economic allocation to quantify the Fish in - Fish-out ratio of major fed aquaculture species. Aquaculture. https://doi.org/10.1016/j.aquaculture.2020.735474

Kolkovski, S., Lazo, J., Leclercq, D., Izquierdo, M., 2009. Fish larvae nutrition and diet: New developments, in: New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management. https://doi.org/10.1533/9781845696474.3.315

Kotzamanis, Y.P., Gisbert, E., Gatesoupe, F.J., Zambonino Infante, J., Cahu, C., 2007. Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to Vibrio anguillarum in European sea bass (Dicentrarchus labrax) larvae. Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology. https://doi.org/10.1016/j.cbpa.2006.12.037

Kuz'mina, V.V., 1996. Influence of age on digestive enzyme activity in some freshwater teleosts. Aquaculture 148, 25–37. https://doi.org/10.1016/S0044-8486(96)01370-1

Lallès, J., 2019. Intestinal alkaline phosphatase in the gastrointestinal tract of fish: biology, ontogeny, and environmental and nutritional modulation. Reviews in Aquaculture 12, 555–581. https://doi.org/10.1111/raq.12340

Lallès, J.P., 2014. Intestinal alkaline phosphatase: Novel functions and protective effects. Nutrition Reviews. https://doi.org/10.1111/nure.12082

Lallès, J.P., 2010. Intestinal alkaline phosphatase: Multiple biological roles in maintenance of intestinal homeostasis and modulation by diet. Nutrition Reviews.

https://doi.org/10.1111/j.1753-4887.2010.00292.x

Lazo, Holt, Arnold, 2000. Ontogeny of pancreatic enzymes in larval red drum Sciaenops ocellatus. Aquaculture Nutrition 6, 183–192. https://doi.org/10.1046/j.1365-2095.2000.00124.x

Lee, C.-S., 2003. Biotechnological advances in finfish hatchery production: a review. Aquaculture 227, 439–458. https://doi.org/10.1016/S0044-8486(03)00522-2

Lemieux, H., Blier, P., Dutil, J.D., 1999. Do digestive enzymes set a physiological limit on growth rate and food conversion efficiency in the Atlantic cod (Gadus morhua)? Fish Physiology and Biochemistry. https://doi.org/10.1023/A:1007791019523

Levine, R.L., Stadtman, E.R., 2001. Oxidative modification of proteins during aging. Experimental Gerontology. https://doi.org/10.1016/S0531-5565(01)00135-8

Li, E., 2002. Chromatin modification and epigenetic reprogramming in mammalian development. Nature Reviews Genetics. https://doi.org/10.1038/nrg887

Lieke, T., Meinelt, T., Hoseinifar, S.H., Pan, B., Straus, D.L., Steinberg, C.E.W., 2020. Sustainable aquaculture requires environmental-friendly treatment strategies for fish diseases. Reviews in Aquaculture. https://doi.org/10.1111/raq.12365

Llorente, I., Fernández-Polanco, J., Baraibar-Diez, E., Odriozola, M.D., Bjørndal, T., Asche, F., Guillen, J., Avdelas, L., Nielsen, R., Cozzolino, M., Luna, M., Fernández-Sánchez, J.L., Luna, L., Aguilera, C., Basurco, B., 2020. Assessment of the economic performance of the seabream and seabass aquaculture industry in the European Union. Marine Policy. https://doi.org/10.1016/j.marpol.2020.103876

Logue, J.A., Howell, B.R., Bell, J.G., Cossins, A.R., 2000. Dietary n-3 long-chain polyunsaturated fatty acid deprivation, tissue lipid composition, ex vivo prostaglandin production, tissue lipid composition, ex vivo prostaglandin production, and stress tolerance in juvenile Dover sole (Solea solea L.). Lipids. https://doi.org/10.1007/s11745-000-0581-3

Ma, H., Cahu, C., Zambonino, J., Yu, H., Duan, Q., Le Gall, M.M., Mai, K., 2005. Activities of selected digestive enzymes during larval development of large yellow croaker (Pseudosciaena crocea). Aquaculture. https://doi.org/10.1016/j.aquaculture.2004.11.032

Machado, M., Engrola, S., Colen, R., Conceição, L.E.C., Dias, J., Costas, B., 2020. Dietary methionine supplementation improves the European seabass (Dicentrarchus labrax) immune status following long-term feeding on fish meal free diets. British Journal of Nutrition 1–29. https://doi.org/10.1017/S0007114520001877

Magoulas, A., Sophronides, K., Patarnello, T., Hatzilaris, E., Zouros, E., 1995. Mitochondrial DNA variation in an experimental stock of gilthead sea bream (*Sparus aurata*). Molecular Marine Biology and Biotechnology Jun;4(2), 110–6.

Magouz, F.I., Dawood, M.A.O., Salem, M.F.I., El-Ghandour, M., Van Doan, H., Mohamed, A.A.I.,

2020. The role of a digestive enhancer in improving the growth performance, digestive enzymes activity, and health condition of Nile tilapia (Oreochromis niloticus) reared under suboptimal temperature. Aquaculture. https://doi.org/10.1016/j.aquaculture.2020.735388

Mahmoud, H.K., Al-Sagheer, A.A., Reda, F.M., Mahgoub, S.A., Ayyat, M.S., 2017a. Dietary curcumin supplement influence on growth, immunity, antioxidant status, and resistance to Aeromonas hydrophila in Oreochromis niloticus. Aquaculture. https://doi.org/10.1016/j.aquaculture.2017.03.043

Mahmoud, H.K., Al-Sagheer, A.A., Reda, F.M., Mahgoub, S.A., Ayyat, M.S., 2017b. Dietary curcumin supplement influence on growth, immunity, antioxidant status, and resistance to Aeromonas hydrophila in Oreochromis niloticus. Aquaculture 475, 16–23. https://doi.org/10.1016/j.aquaculture.2017.03.043

Mandal, M.N.A., Patlolla, J.M.R., Zheng, L., Agbaga, M.-P., Tran, J.-T.A., Wicker, L., Kasus-Jacobi, A., Elliott, M.H., Rao, C. V., Anderson, R.E., 2009. Curcumin protects retinal cells from light-and oxidant stress-induced cell death. Free Radical Biology and Medicine 46, 672–679. https://doi.org/10.1016/j.freeradbiomed.2008.12.006

Massa, F., 2017. Aquaculture in the Mediterranean and the Black Sea: a Blue Growth perspective, in: Handbook on the Economics and Management of Sustainable Oceans. Edward Elgar Publishing, pp. 93–123. https://doi.org/10.4337/9781786430724.00013

Mastoraki, M., Ferrándiz, P.M., Vardali, S.C., Kontodimas, D.C., Kotzamanis, Y.P., Gasco, L., Chatzifotis, S., Antonopoulou, E., 2020. A comparative study on the effect of fish meal substitution with three different insect meals on growth, body composition and metabolism of European sea bass (Dicentrarchus labrax L.). Aquaculture 735511. https://doi.org/10.1016/j.aquaculture.2020.735511

Mata-Sotres, J.A., Martínez-Rodríguez, G., Pérez-Sánchez, J., Sánchez-Vázquez, F.J., Yúfera, M., 2015. Daily rhythms of clock gene expression and feeding behavior during the larval development in gilthead seabream, Sparus aurata. Chronobiology International. https://doi.org/10.3109/07420528.2015.1058271

Mata-Sotres, J.A., Moyano, F.J., Martínez-Rodríguez, G., Yúfera, M., 2016. Daily rhythms of digestive enzyme activity and gene expression in gilthead seabream (Sparus aurata) during ontogeny. Comparative Biochemistry and Physiology -Part A : Molecular and Integrative Physiology 197. https://doi.org/10.1016/j.cbpa.2016.03.010

Matsunari, H., Hashimoto, H., Iwasaki, T., Oda, K., Masuda, Y., Imaizumi, H., Teruya, K., Furuita, H., Yamamoto, T., Hamada, K., Mushiake, K., 2013. Effect of feeding rotifers enriched with taurine on the growth and survival of larval amberjack Seriola dumerili. Fisheries Science. https://doi.org/10.1007/s12562-013-0657-y

Matzke, M.A., Birchler, J.A., 2005. RNAi-mediated pathways in the nucleus. Nature Reviews Genetics. https://doi.org/10.1038/nrg1500

Merad-Boudia, M., Nicole, A., Santiard-Baron, D., Saillé, C., Ceballos-Picot, I., 1998. Mitochondrial impairment as an early event in the process of apoptosis induced by glutathione depletion in neuronal cells: Relevance to Parkinson's disease. Biochemical Pharmacology. https://doi.org/10.1016/S0006-2952(97)00647-3

Mesquita, C.S., Oliveira, R., Bento, F., Geraldo, D., Rodrigues, J. V., Marcos, J.C., 2014. Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. Analytical Biochemistry. https://doi.org/10.1016/j.ab.2014.04.034

Metges, C.C., Görs, S., Lang, I.S., Hammon, H.M., Brüssow, K.-P., Weitzel, J.M., Nürnberg, G., Rehfeldt, C., Otten, W., 2014. Low and High Dietary Protein:Carbohydrate Ratios during Pregnancy Affect Materno-Fetal Glucose Metabolism in Pigs. The Journal of Nutrition 144, 155–163. https://doi.org/10.3945/jn.113.182691

Midhun, S.J., Arun, D., Edatt, L., Sruthi, M. V., Thushara, V. V., Oommen, O. V., Sameer Kumar, V.B., Divya, L., 2016. Modulation of digestive enzymes, GH, IGF-1 and IGF-2 genes in the teleost, Tilapia (*Oreochromis mossambicus*) by dietary curcumin. Aquaculture International 24, 1277–1286. https://doi.org/10.1007/s10499-016-9984-1

Ming, J., Ye, J., Zhang, Y., Yang, X., Shao, X., Qiang, J., Xu, P., 2019. Dietary optimal reduced glutathione improves innate immunity, oxidative stress resistance and detoxification function of grass carp (Ctenopharyngodon idella) against microcystin-LR. Aquaculture. https://doi.org/10.1016/j.aquaculture.2018.09.014

Molosse, V., Souza, C.F., Baldissera, M.D., Glombowsky, P., Campigotto, G., Cazaratto, C.J., Stefani, L.M., da Silva, A.S., 2019. Diet supplemented with curcumin for nursing lambs improves animal growth, energetic metabolism, and performance of the antioxidant and immune systems. Small Ruminant Research 170, 74–81. https://doi.org/10.1016/j.smallrumres.2018.11.014

Moretti, A., Fernandez-Criado, M.P., Vetillart, R., 2005. Manual on Hatchery Production of Seabass and Gilthead Seabream, volume 2. ed. FAO, Rome.

Mouler Rechtman, M., Har-Noy, O., Bar-Yishay, I., Fishman, S., Adamovich, Y., Shaul, Y., Halpern, Z., Shlomai, A., 2010. Curcumin inhibits hepatitis B virus via down-regulation of the metabolic coactivator PGC-1α. FEBS Letters 584, 2485–2490. https://doi.org/10.1016/j.febslet.2010.04.067

Moyano, F.J., Díaz, M., Alarcón, F.J., Sarasquete, M.C., 1996. Characterization of digestive enzyme activity during larval development of gilthead seabream (Sparus aurata). Fish

Physiology and Biochemistry. https://doi.org/10.1007/BF01875591

Mozsár, A., Boros, G., Sály, P., Antal, L., Nagy, S.A., 2015. Relationship between Fulton's condition factor and proximate body composition in three freshwater fish species. Journal of Applied Ichthyology 31, 315–320. https://doi.org/10.1111/jai.12658

Navarro-Guillén, C., Moyano, F.J., Yúfera, M., 2015. Diel food intake and digestive enzyme production patterns in *Solea senegalensis* larvae. Aquaculture 435, 33–42. https://doi.org/10.1016/j.aquaculture.2014.09.017

Naz, M., 2009. Ontogeny of biochemical phases of fertilized eggs and Yolk sac larvae of gilthead seabream (Sparus aurata L.). Turkish Journal of Fisheries and Aquatic Sciences.

Novelli, B., Otero-Ferrer, F., Diaz, M., Socorro, J.A., Caballero, M.J., Domínguez, L.M., Moyano, F.J., 2016. Digestive biochemistry as indicator of the nutritional status during early development of the long snouted seahorse (Hippocampus reidi). Aquaculture. https://doi.org/10.1016/j.aquaculture.2016.06.037

Nya, E.J., Austin, B., 2011. Development of immunity in rainbow trout (Oncorhynchus mykiss, Walbaum) to Aeromonas hydrophila after the dietary application of garlic. Fish and Shellfish Immunology. https://doi.org/10.1016/j.fsi.2011.01.008

OECD, 2020. Aquaculture production (indicator). https://doi.org/10.1787/d00923d8-en

Oozeki, Y., Bailey, K.M., 1995. Ontogenetic development of digestive enzyme activities in larval walleye pollock, Theragra chalcogramma. Marine Biology. https://doi.org/10.1007/BF00348930

Osse, J.W.M., Van Den Boogaart, J.G.M., Van Snik, G.M.J., Van Der Sluys, L., 1997. Priorities during early growth of fish larvae, in: Aquaculture. https://doi.org/10.1016/S0044-8486(97)00126-9

Ott, M., Gogvadze, V., Orrenius, S., Zhivotovsky, B., 2007. Mitochondria, oxidative stress and cell death. Apoptosis 12, 913–922. https://doi.org/10.1007/s10495-007-0756-2

Pantazis, P.A., Benekos, G., Papadomichelakis, G., 2014. Early-weaning diets for gilthead sea bream (*Sparus aurata L.*) and their potential use in Hellenic marine fish hatcheries. Aquaculture International 22, 1621–1636. https://doi.org/10.1007/s10499-014-9769-3

Paradies, G., Petrosillo, G., Paradies, V., Ruggiero, F.M., 2011. Mitochondrial dysfunction in brain aging: Role of oxidative stress and cardiolipin. Neurochemistry International. https://doi.org/10.1016/j.neuint.2010.12.016

Parker, R.W.R., Blanchard, J.L., Gardner, C., Green, B.S., Hartmann, K., Tyedmers, P.H., Watson, R.A., 2018. Fuel use and greenhouse gas emissions of world fisheries. Nature Climate Change. https://doi.org/10.1038/s41558-018-0117-x

Parma, L., Pelusio, N.F., Gisbert, E., Esteban, M.A., D'Amico, F., Soverini, M., Candela, M.,

Dondi, F., Gatta, P.P., Bonaldo, A., 2020. Effects of rearing density on growth, digestive conditions, welfare indicators and gut bacterial community of gilthead sea bream (Sparus aurata, L. 1758) fed different fishmeal and fish oil dietary levels. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.734854

Patel, M.S., Srinivasan, M., 2002. Metabolic Programming: Causes and Consequences. Journal of Biological Chemistry 277, 1629–1632. https://doi.org/10.1074/jbc.R100017200

Perera, E., Yúfera, M., 2017. Effects of soybean meal on digestive enzymes activity, expression of inflammation-related genes, and chromatin modifications in marine fish (Sparus aurata L.) larvae. Fish Physiology and Biochemistry. https://doi.org/10.1007/s10695-016-0310-7

Péres, A., Cahu, C.L., Infante, J.L.Z., Le Gall, M.M., Quazuguel, P., 1996. Amylase and trypsin responses to intake of dietary carbohydrate and protein depend on the developmental stage in sea bass (Dicentrarchus labrax) larvae. Fish Physiology and Biochemistry. https://doi.org/10.1007/BF01875574

Pinto, W., Engrola, S., Conceição, L.E.C., 2018. Towards an early weaning in Senegalese sole: A historical review. Aquaculture. https://doi.org/10.1016/j.aquaculture.2018.06.077

Platel, K., Srinivasan, K., 2000. Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. Nahrung/Food 44, 42–46. https://doi.org/10.1002/(SICI)1521-3803(20000101)44:1<42::AID-FOOD42>3.0.CO;2-D

Poore, J., Nemecek, T., 2018. Reducing food's environmental impacts through producers and consumers. Science. https://doi.org/10.1126/science.aaq0216

Prasad, S., Gupta, S.C., Tyagi, A.K., Aggarwal, B.B., 2014. Curcumin, a component of golden spice: From bedside to bench and back. Biotechnology Advances 32, 1053–1064. https://doi.org/10.1016/j.biotechadv.2014.04.004

Priya, R., Prathapan, A., Raghu, K.G., Menon, A.N., 2012. Chemical composition and in vitro antioxidative potential of essential oil isolated from Curcuma longa L. leaves. Asian Pacific Journal of Tropical Biomedicine. https://doi.org/10.1016/S2221-1691(12)60298-6

Rad, F., 2007. Evaluation of the Sea Bass and Sea Bream Industry in the Mediterranean, with Emphasis on Turkey, in: Species and System Selection for Sustainable Aquaculture. https://doi.org/10.1002/9780470277867.ch28

Rad, F., Köksal, G., 2000. An overview of aquaculture in Turkey: With emphasis on sea bass and sea bream. Aquaculture Economics and Management. https://doi.org/10.1080/13657300009380271

Rader, B.A., 2017. Alkaline phosphatase, an unconventional immune protein. Frontiers in Immunology. https://doi.org/10.3389/fimmu.2017.00897

Ran, C., Huang, L., Hu, J., Tacon, P., He, S., Li, Z., Wang, Y., Liu, Z., Xu, L., Yang, Y., Zhou, Z.,

2016. Effects of dietary live and heat-inactive baker's yeast on growth, gut health, and disease resistance of Nile tilapia under high rearing density. Fish and Shellfish Immunology. https://doi.org/10.1016/j.fsi.2016.07.001

Reda, F.M., El-Saadony, M.T., Elnesr, S.S., Alagawany, M., Tufarelli, V., 2020. Effect of dietary supplementation of biological curcumin nanoparticles on growth and carcass traits, antioxidant status, immunity and caecal microbiota of Japanese quails. Animals. https://doi.org/10.3390/ani10050754

Regnier, E., Bayramoglu, B., 2017. Competition between farmed and wild fish: The French sea bass and sea bream markets. Aquaculture Economics and Management. https://doi.org/10.1080/13657305.2016.1189012

Reik, W., Dean, W., Walter, J., 2001. Epigenetic reprogramming in mammalian development. Science. https://doi.org/10.1126/science.1063443

Ribeiro, L., Zambonino-Infante, J.L., Cahu, C., Dinis, M.T., 1999. Development of digestive enzymes in larvae of Solea senegalensis, Kaup 1858. Aquaculture. https://doi.org/10.1016/S0044-8486(99)00180-5

Rice-Evans, C., Miller, N.J., 1994. Total antioxidant status in plasma and body fluids. Methods in Enzymology. https://doi.org/10.1016/0076-6879(94)34095-1

Ricker, W.E., Parker, R.A., 1960. Handbook of Computations for Biological Statistics of Fish Populations. The Journal of Wildlife Management. https://doi.org/10.2307/3796760

Rocha, F., Dias, J., Engrola, S., Gavaia, P., Geurden, I., Dinis, M.T., Panserat, S., 2015. Glucose metabolism and gene expression in juvenile zebrafish (*Danio rerio*) challenged with a high carbohydrate diet: effects of an acute glucose stimulus during late embryonic life. British Journal of Nutrition 113, 403–413. https://doi.org/10.1017/S0007114514003869

Rocha, F., Dias, J., Geurden, I., Dinis, M.T., Panserat, S., Engrola, S., 2016. Dietary glucose stimulus at larval stage modifies the carbohydrate metabolic pathway in gilthead seabream (Sparus aurata) juveniles: An in vivo approach using 14C-starch. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 201, 189–199. https://doi.org/10.1016/j.cbpa.2016.07.016

Rodrigues, A.C.M., Gravato, C., Quintaneiro, C., Bordalo, M.D., Golovko, O., Žlábek, V., Barata, C., Soares, A.M.V.M., Pestana, J.L.T., 2017. Exposure to chlorantraniliprole affects the energy metabolism of the caddisfly Sericostoma vittatum. Environmental Toxicology and Chemistry. https://doi.org/10.1002/etc.3684

Romero-Romero, S., Yúfera, M., 2012. Contribution of gut content to the nutritional value of Brachionus plicatilis used as prey in larviculture. Aquaculture. https://doi.org/10.1016/j.aquaculture.2012.08.011

Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., Boglione, C., 2013. Feeding behaviour and digestive physiology in larval fish: Current knowledge, and gaps and bottlenecks in research. Reviews in Aquaculture. https://doi.org/10.1111/raq.12010

Röös, E., Bajželj, B., Smith, P., Patel, M., Little, D., Garnett, T., 2017. Protein futures for Western Europe: potential land use and climate impacts in 2050. Regional Environmental Change. https://doi.org/10.1007/s10113-016-1013-4

Rotllant, G., Moyano, F.J., Andrés, M., Díaz, M., Estévez, A., Gisbert, E., 2008. Evaluation of fluorogenic substrates in the assessment of digestive enzymes in a decapod crustacean *Maja brachydactyla* larvae. Aquaculture 282, 90–96. https://doi.org/10.1016/j.aquaculture.2008.06.004

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 Physiology and Biochemistry. https://doi.org/10.1007/s10695-005-0630-5

Saeij, J.P.J., Van Muiswinkel, W.B., Van De Meent, M., Amaral, C., Wiegertjes, G.F., 2003. Different capacities of carp leukocytes to encounter nitric oxide-mediated stress: A role for the intracellular reduced glutathione pool. Developmental and Comparative Immunology. https://doi.org/10.1016/S0145-305X(02)00158-1

Sala, R., Santamaría, C.A., Crespo, S., 2005. Growth of organ systems of Dentex dentex (L) and Psetta maxima (L) during larval development. Journal of Fish Biology. https://doi.org/10.1111/j.0022-1112.2005.00580.x

Sanz, Y., Toldra, F., 2002. Purification and Characterization of an Arginine Aminopeptidase from Lactobacillus sakei. Applied and Environmental Microbiology 68, 1980–1987. https://doi.org/10.1128/AEM.68.4.1980-1987.2002

Shi, X., Zheng, Z., Li, J., Xiao, Z., Qi, W., Zhang, A., Wu, Q., Fang, Y., 2015. Curcumin inhibits Aβ-induced microglial inflammatory responses in vitro: involvement of ERK1/2 and p38 signaling pathways. Neurosci. Lett. 594, 105–110.

Shigenaga, M.K., Hagen, T.M., Ames, B.N., 1994. Oxidative damage and mitochondrial decay in aging. Proceedings of the National Academy of Sciences of the United States of America. https://doi.org/10.1073/pnas.91.23.10771

Sohal, R.S., Mockett, R.J., Orr, W.C., 2002. Mechanisms of aging: An appraisal of the oxidative stress hypothesis. Free Radical Biology and Medicine. https://doi.org/10.1016/S0891-5849(02)00886-9

Srivastava, R.M., Singh, S., Dubey, S.K., Misra, K., Khar, A., 2011. Immunomodulatory and therapeutic activity of curcumin. International Immunopharmacology. https://doi.org/10.1016/j.intimp.2010.08.014

Stadtman, E.R., 2006. Protein oxidation and aging. Free Radical Research. https://doi.org/10.1080/10715760600918142

STECF, 2018. The economic performance of the EU aquaculture sector. Publications Office of the European Union. JRC scient, 451 pp. https://doi.org/10.2760/638363

STEFC, 2015. Scientific, Technical and Economic Committee for Fisheries (STECF) – The 2015 Annual Economic Report on the EU Fishing Fleet (STECF-15-07), Publications Office of the European Union. https://doi.org/10.2788/23331

Stephensen, E., Sturve, J., Förlin, L., 2002. Effects of redox cycling compounds on glutathione content and activity of glutathione-related enzymes in rainbow trout liver. Comparative Biochemistry and Physiology - C Toxicology and Pharmacology. https://doi.org/10.1016/S1532-0456(02)00129-1

Sunde, J., Taranger, G.L., Rungruangsak-Torrissen, K., 2001. Digestive protease activities and free amino acids in white muscle as indicators for feed conversion efficiency and growth rate in Atlantic salmon (Salmo salar L.). Fish Physiology and Biochemistry. https://doi.org/10.1023/A:1023233024001

Suzer, C., Kamaci, H.O., Çoban, D., Saka, Ş., Firat, K., Özkara, B., Özkara, A., 2007. Digestive enzyme activity of the red porgy (Pagrus pagrus, L.) during larval development under culture conditions. Aquaculture Research. https://doi.org/10.1111/j.1365-2109.2007.01841.x

Takagi, S., Murata, H., Goto, T., Endo, M., Yamashita, H., Ukawa, M., 2008. Taurine is an essential nutrient for yellowtail Seriola quinqueradiata fed non-fish meal diets based on soy protein concentrate. Aquaculture. https://doi.org/10.1016/j.aquaculture.2008.05.012

Teodósio, R., Engrola, S., Colen, R., Masagounder, K., Aragão, C., 2020. Optimizing diets to decrease environmental impact of Nile tilapia (Oreochromis niloticus) production. Aquaculture Nutrition. https://doi.org/10.1111/anu.13004

Tietze, F., 1969. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: Applications to mammalian blood and other tissues. Analytical Biochemistry. https://doi.org/10.1016/0003-2697(69)90064-5

Toledo-Cuevas, E.M., Moyano López, F.J., Ramírez, D.T., Strüssmann, C.A., Álvarez-González, C.A., Martínez-Chávez, C.C., Martínez-Palacios, C.A., 2011. Development of digestive biochemistry in the initial stages of three cultured Atherinopsids. Aquaculture Research 42, 776–786. https://doi.org/10.1111/j.1365-2109.2011.02853.x

Trujillo, P., Piroddi, C., Jacquet, J., 2012. Fish farms at Sea: The ground truth from Google Earth. PLoS ONE. https://doi.org/10.1371/journal.pone.0030546

Türk, G., Çeribaşı, A.O., Şimşek, Ü.G., Çeribaşı, S., Güvenç, M., Özer Kaya, Ş., Çiftçi, M., Sönmez, M., Yüce, A., Bayrakdar, A., Yaman, M., Tonbak, F., 2016. Dietary rosemary oil

alleviates heat stress-induced structural and functional damage through lipid peroxidation in the testes of growing Japanese quail. Animal Reproduction Science 164, 133–143. https://doi.org/10.1016/j.anireprosci.2015.11.021

Uscanga-Martínez, A., Perales-García, N., Álvarez-González, C.A., Moyano, F.J., Tovar-Ramírez, D., Gisbert, G.E., Márquez-Couturier, G., Contreras-Sánchez, W.M., Arias-Rodríguez, L., Indy, J.R., 2011. Changes in digestive enzyme activity during initial ontogeny of bay snook Petenia splendida. Fish Physiology and Biochemistry 37, 667–680. https://doi.org/10.1007/s10695-011-9467-2

Vadstein, O., Bergh, Ø., Gatesoupe, F.J., Galindo-Villegas, J., Mulero, V., Picchietti, S., Scapigliati, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., De Schryver, P., Bossier, P., 2013. Microbiology and immunology of fish larvae. Reviews in Aquaculture. https://doi.org/10.1111/j.1753-5131.2012.01082.x

Vadstein, O., Mo, T.A., Bergh, Ø., 2007. Microbial Interactions, Prophylaxis and Diseases, in: Culture of Cold-Water Marine Fish. https://doi.org/10.1002/9780470995617.ch3

Vagner, M., Zambonino-Infante, J.L., Mazurais, D., 2019. Fish facing global change: are early stages the lifeline? Marine Environmental Research. https://doi.org/10.1016/j.marenvres.2019.04.005

Valente, L.M.P., Moutou, K.A., Conceição, L.E.C., Engrola, S., Fernandes, J.M.O., Johnston, I.A., 2013. What determines growth potential and juvenile quality of farmed fish species? Reviews in Aquaculture. https://doi.org/10.1111/raq.12020

Van Der Toorn, M., Kauffman, H.F., Van Der Deen, M., Slebos, D.J., Koëter, G.H., Gans, R.O.B., Bakker, S.J.L., 2007. Cyclosporin A-induced oxidative stress is not the consequence of an increase in mitochondrial membrane potential. FEBS Journal. https://doi.org/10.1111/j.1742-4658.2007.05827.x

Vaquerizas, J.M., Kummerfeld, S.K., Teichmann, S.A., Luscombe, N.M., 2009. A census of human transcription factors: Function, expression and evolution. Nature Reviews Genetics. https://doi.org/10.1038/nrg2538

Vorlaphim, 2011. Influence of Dietary Curcumin on Rumen Fermentation, Macronutrient Digestion and Nitrogen Balance in Beef Cattle. American Journal of Agricultural and Biological Sciences 6, 7–11. https://doi.org/10.3844/ajabssp.2011.7.11

Wang, J.L., Meng, X. lin, Lu, R. hua, Wu, C., Luo, Y.T., Yan, X., Li, X.J., Kong, X.H., Nie, G.X., 2015. Effects of Rehmannia glutinosa on growth performance, immunological parameters and disease resistance to Aeromonas hydrophila in common carp (Cyprinus carpio L.). Aquaculture. https://doi.org/10.1016/j.aquaculture.2014.10.004

Wang, X. xi, Yuan, Y., Li, C. chen, Zhou, F., Jin, M., Sun, P., Zhu, T. ting, Ding, X. yan, Zhou, Q.

cun, 2020. Partial substitution of fish meal with soy protein concentrate in commercial diets for juvenile swimming crab, Portunus trituberculatus. Animal Feed Science and Technology. https://doi.org/10.1016/j.anifeedsci.2019.114290

Wu, R.X., Hong, W.S., Zhang, Q.Y., Chen, S.X., 2009. Comparative enzyme activities of the intestinal brush border membranes of the herbivorous mudskipper Boleophthalmus pectinirostris and the carnivorous Chinese black sleeper Bostrichthys sinensis. Journal of Applied Ichthyology. https://doi.org/10.1111/j.1439-0426.2009.01302.x

Xiao, W., Jiang, W., Feng, L., Liu, Y., Wu, P., Jiang, J., Zhang, Y., Zhou, X., 2017. Supplementation of enzyme-treated soy protein saves dietary protein and promotes digestive and absorptive ability referring to TOR signaling in juvenile fish. Fish Physiology and Biochemistry. https://doi.org/10.1007/s10695-017-0400-1

Xu, C., Li, X.F., Tian, H.Y., Jiang, G.Z., Liu, W. Bin, 2016. Feeding rates affect growth, intestinal digestive and absorptive capabilities and endocrine functions of juvenile blunt snout bream Megalobrama amblycephala. Fish Physiology and Biochemistry. https://doi.org/10.1007/s10695-015-0169-z

Xu, H., Turkmen, S., Rimoldi, S., Terova, G., Zamorano, M.J., Afonso, J.M., Sarih, S., Fernández-Palacios, H., Izquierdo, M., 2019. Nutritional intervention through dietary vegetable proteins and lipids to gilthead sea bream (Sparus aurata) broodstock affects the offspring utilization of a low fishmeal/fish oil diet. Aquaculture. https://doi.org/10.1016/j.aquaculture.2019.734402

Yadav, G., Meena, D.K., Sahoo, A.K., Das, B.K., Sen, R., 2020. Effective valorization of microalgal biomass for the production of nutritional fish-feed supplements. Journal of Cleaner Production. https://doi.org/10.1016/j.jclepro.2019.118697

Yin, G., Ardó, L., Thompson, K.D., Adams, A., Jeney, Z., Jeney, G., 2009. Chinese herbs (Astragalus radix and Ganoderma lucidum) enhance immune response of carp, Cyprinus carpio, and protection against Aeromonas hydrophila. Fish and Shellfish Immunology. https://doi.org/10.1016/j.fsi.2008.08.015

Young, I.S., Woodside, J. V., 2001. Antioxidants in health and disease. Journal of Clinical Pathology. https://doi.org/10.1136/jcp.54.3.176

Yúfera, M., Darias, M.J., 2007. The onset of exogenous feeding in marine fish larvae. Aquaculture. https://doi.org/10.1016/j.aquaculture.2007.04.050

Yúfera, M., Fernández-Díaz, C., Pascual, E., 2005. Food microparticles for larval fish prepared by internal gelation, in: Aquaculture. https://doi.org/10.1016/j.aquaculture.2005.04.026

Yúfera, M., Mata-Sotres, J.A., Navarro-Guillén, C., Moyano, F.J., Martínez-Rodríguez, G., 2016. Potential effect of increasing the water content in the digestibility of microdiets for fish larvae. Aquaculture Nutrition. https://doi.org/10.1111/anu.12336

Zambonino-Infante, J.L., Gisbert, E., Sarasquete, C., Navarro, I., Gutiérrez, J., Cahu, C.L., 2008. Feeding and Digestive Functions in Fishes., in: In: Cyrino, J.E.P., Bureau, D., Kapoor, B.G. (Eds.), CRC Press,. pp. 281–348.

Zambonino-Infante, J.L., Panserat, S., Servili, A., Mouchel, O., Madec, L., Mazurais, D., 2019. Nutritional programming by dietary carbohydrates in European sea bass larvae: Not always what expected at juvenile stage. Aquaculture 501, 441–447. https://doi.org/10.1016/j.aquaculture.2018.11.056

Zambonino Infante, J.L., Cahu, C.L., 2007. Dietary modulation of some digestive enzymes and Metabolic processes in developing marine fish: Applications to diet formulation. Aquaculture. https://doi.org/10.1016/j.aquaculture.2007.04.032

Zambonino Infante, J.L., Cahu, C.L., 2001. Ontogeny of the gastrointestinal tract of marine fish larvae, in: Comparative Biochemistry and Physiology - C Toxicology and Pharmacology. https://doi.org/10.1016/S1532-0456(01)00274-5

Zhang, N., 2015. Epigenetic modulation of DNA methylation by nutrition and its mechanisms in animals. Animal Nutrition 1, 144–151. https://doi.org/10.1016/j.aninu.2015.09.002

Zouiten, D., Khemis, I. Ben, Besbes, R., Cahu, C., 2008. Ontogeny of the digestive tract of thick lipped grey mullet (Chelon labrosus) larvae reared in “mesocosms.” Aquaculture. https://doi.org/10.1016/j.aquaculture.2008.03.039

7. Annexes

Table 3.2.1. Enzymatic activity levels of trypsin, chymotrypsin, aminopeptidase, trypsin/chymotrypsin ratio, amylase, 4-C like and 18-C like lipases, and alkaline phosphatase (RFU/mg DW), in *S. aurata* larvae, measured at 10, 24 and 31 DAH.

	Treatments		
	CTRL	**LOW**	**HIGH**
Trypsin			
10 DAH	3.93E+04 ± 1.62E+04	3.20E+04 ± 1.58E+04	5.90E+04 ± 1.23E+04
24 DAH	5.04E+03 ± 1.77E+03	4.32E+03 ± 2.34E+03	7.85E+04 ± 1.28E+04
31 DAH	5.90E+04 ± 1.23E+04	6.20E+03 ± 2.21E+03	1.03E+05 ± 2.50E+04
Chymotrypsin			
10 DAH	not detected	not detected	not detected
24 DAH	not detected	not detected	not detected
31 DAH	5.52E+04 ± 1.74E+04	7.31E+04 ± 1.73E+04	9.53E+04 ± 1.76E+04
Aminopeptidase			
10 DAH	not detected	not detected	not detected
24 DAH	not detected	not detected	not detected
31 DAH	3.00E+03 ± 1.47E+03	2.17E+03 ± 5.94E+02	2.72E+03 ± 9.41E+02
Trypsin/Chymotrypsin ratio			
31 DAH	1.07 ± 0.19	1.09 ± 0.94	1.07 ± 0.86
Amylase			
10 DAH	not detected	not detected	not detected
24 DAH	7.54E+04 ± 2.30E+04	6.84E+04 ± 2.06E+04	6.84E+04 ± 1.60E+04
31 DAH	4.35E+04 ± 1.79E+04	3.38E+04 ± 1.53E+04	3.75E+04 ± 1.14E+04
4C-Like lipase			
10 DAH	1.55E+04 ± 3.48E+03	1.72E+04 ± 5.62E+03	1.18E+04 ± 3.33E+03
24 DAH	3.77E+03 ± 5.74E+02	2.79E+03 ± 1.03E+03	2.93E+03 ± 7.83E+02
31 DAH	4.30E+03 ± 6.11E+02	3.96E+03 ± 6.80E+02	4.57E+03 ± 7.73E+02
18C-Like lipase			
10 DAH	not detected	not detected	not detected
24 DAH	6.95E+04 ± 7.59E+03	4.05E+04 ± 1.37E+04	3.99E+04 ± 8.82E+03
31 DAH	2.86E+04 ± 5.43E+03	2.56E+04 ± 1.01E+04	2.63E+04 ± 5.90E+03
Alkaline phospatase			
10 DAH	not detected	not detected	not detected
24 DAH	1.63E+05 ± 4.99E+04	1.19E+05 ± 3.45E+04	1.53E+05 ± 5.10E+04
31 DAH	2.41E+05 ± 8.66E+04	2.37E+05 ± 5.31E+04	2.22E+05 ± 7.21E+04

Results are means ± SD.

Table 3.3.1. Gut fullness (area/TL) of *S. aurata* larvae at 5, 6, 8, 12, 16, 20, 23, 28 DAH fed experimental diets CTRL, LOW and HIGH.

	CONTROL	**LOW**	**HIGH**
5 DAH	0.001 ± 0.001	0.002 ± 0.001	0.001 ± 0.001
6 DAH	0.001 ± 0.001	0.022 ± 0.002	0.015 ± 0.001
8 DAH	0.003 ± 0.003	0.004 ± 0.003	0.004 ± 0.003
12 DAH	0.009 ± 0.006	0.012 ± 0.008	0.024 ± 0.016
16 DAH	0.025 ± 0.013	0.019 ± 0.011	0.018 ± 0.014
20 DAH	0.021 ± 0.014	0.016 ± 0.010	0.012 ± 0.014
23 DAH	0.020 ± 0.012	0.022 ± 0.010	0.033 ± 0.016
28 DAH	0.027 ± 0.011	0.024 ± 0.011	0.020 ± 0.011

Results are means ± SD.