A balanced amino acid diet improves *Diplodus sargus* larval quality and reduces nitrogen excretion

Saavedra, M.[1]*, Pousão-Ferreira, P.[1], Yúfera, M.[3] Dinis, M.T.[2] and Conceição, L.E.C.[2]

[1]Instituto Nacional de Investigação Agrária e das Pescas (INIAP/IPIMAR-CRIPSul), Av. 5 de Outubro, 8700-305 Olhão, Portugal

[2]CCMAR, Universidade do Algarve, Campus Gambelas, 8005-139, Faro, Portugal

[3]Instituto de Ciencias Marinas Andalucia (CSIC), Apartado Oficial,11510 Puerto Real, Spain

**Corresponding author. Tel.: +351 289 71 53 46, Fax.: +351 289 71 55 79*

E-mail address: margarida.saavedra@gmail.com

Abstract

Fish larvae present high amino acid requirements due to their high growth rate. Maximizing this growth rate depends on providing a balanced amino acid diet which can fulfil larval amino acid nutritional needs. In this study two experimental microencapsulated casein diets were tested: one presenting a balanced amino acid profile and another presenting an unbalanced amino acid profile. A control diet, live feed based, was also tested. Trials were performed with larvae from 1 to 25 DAH. Microencapsulated diets were introduced at 8 DAH in co-feeding with live feed and at 15 DAH larvae were fed the microencapsulated diets alone. Results showed a higher survival for the control group (8.6 ±1.3% versus 4.2 ± 0.6% and 3.2 ± 1.8%) although

dry weight and growth were similar in all treatments. The proportion of deformed larvae as well as the ammonia excretion was lower in the group fed a balanced diet than in the unbalanced or control groups (38.3% deformed larvae in control, 30% in larvae fed unbalanced diet and 20% on balanced diet group). Furthermore, larvae fed the microencapsulated diets presented higher DHA and ARA levels. This study demonstrates that dietary amino acid profile may play an important role in larval quality. It also shows that balanced microencapsulated diets may improve some of the performance criteria, such as skeletal deformities, compared to live feeds.

Key words: *Diplodus sargus*, amino acids, balanced diet, ammonia, deformities, microencapsulated diet

Introduction

White seabream (*Diplodus sargus*) is considered to be a promising new species to fish farming in Southern Europe, having a high market price and demand (Pousão-Ferreira et al., 2001, Ozorio et al., 2006, Santos et al., 2006). It is also involved in restocking programmes in the southern coast of Portugal, where landings decreased from 200.3 t to 75.2 t between 1987 and 2004 (Santos et al., 2006).

Saavedra et al. (2006) reported some promising results of *Diplodus sargus* larvae rearing, indicating a higher growth rate at the larval stage when compared to *Sparus aurata*. However, when approaching the juvenile stage, a decrease on the growth rate (Cejas et al., 2003) and the presence of several skeletal deformities, especially at the dorsal column, are bottlenecks to this species farming (Saavedra et al., 2006). Recent

studies suggest that the nutritional composition of the diet may play an important role in the frequency of skeletal deformities (Cahu et al., 2003) and the deficiency of some amino acids may increase this percentage (Akiyama et al., 1986). Skeletal deformities can have an economical impact as they can affect both size and shape of the fish therefore decreasing their economical value (Favaloro and Mazzola, 2000; Boglione et al., 2001). They can also make commercialization difficult as the consumers tend to choose fish with standard shape (Koumoundourous et al., 1997, Boglione et al., 2001).

Ten amino acids (AA) are considered indispensable for normal fish growth (Wilson, 1989) and the AA profile of the diet affects larval growth (Conceição et al., 2003). In order to maximize larval growth rate the AA profile of the diet should be as close as possible to the larval AA requirements (Akiyama et al., 1995, Conceição et al., 2003). In fact, juvenile rainbow trout, in presence of several AA diets, selected preferentially balanced diets (Yamamoto et al., 2000). Balanced diets increase protein synthesis as well as decrease nitrogen excretion (Aragão et al., 2004a).

Manipulation of rotifers and *Artemia* AA profiles is difficult (Aragão et al., 2004b) and a balanced AA diet can be better achieved using a microencapsulated feed. However, weaning Sparidae larvae in early stages can compromise survival and growth as there is still a high dependence on live feed (Yúfera et al., 1996, Fernández-Díaz and Yúfera, 1997). Yúfera et al. (1999, 2002) have developed cross-linked casein-walled capsules and showed this type of microencapsulated diet was able to substitute with some success live food in *Sparus aurata* early larval stages. During the first feeding days a microencapsulated diet used in co-feeding with rotifers can increase the enzymatic

capacity to digest the inert diet (Yúfera et al., 1999, 2002). This suggests that free AA in the tissues of the live feed may be important in stimulating the release of digestive enzymes and enhancing the ingestion rates by affecting the attractability of the diets (Cahu and Zambonino Infante, 1995).

The purpose of this study was to determine if a balanced AA diet would promote growth as well as high quality larvae. The balanced diet was given to the fish larvae in the form of cross-linked casein-walled capsules.

Materials and methods

Husbandry and experimental set-up

This study was carried out at the Aquaculture Research Station of INIAP/IPIMAR, in Olhão, South of Portugal during 2006. *D. sargus* eggs were obtained from a mix of wild and farmed broodstock consisting of 38 fish with an average weight of 869.2 g ± 319.8 g and tank density of 4 kg/m^3. After collected from the incubators, first feeding larvae were transferred to 200l conical cylindrical fibreglass tanks at a density of 80 larvae/ l. Before entering the tank water passed through a biological filter and then through a mechanical and sterilized by a UV filter. The system worked in a semi-closed circuit and water temperature was maintained at 19.1 ± 0.7 º C, oxygen at 6.5 ± 0.4 mg /l and salinity at 37 ± 1 ppt. Water flow started at 0.4 l /min and then was slowly increased with larvae age until a maximum of 1 l /min, at 12 DAH. Photoperiod was 24 hours light. The trial lasted 25 days and three treatments, randomly distributed, were used in triplicate: larvae fed a balanced amino acid microencapsulated diet, larvae fed an unbalanced amino acid microencapsulated diet and a control fed live feed.

Cross-linked casein-walled capsules formulation

Cross-linked casein-walled capsules were used in this study due to the lower free amino acid leaching when compared to other kind of inert diets (Yúfera et al., 2002). To formulate the balanced diet, indispensable amino acid profiles from larval carcass were used (Saavedra et al., 2006) as they are commonly used as a good indicator of fish amino acids requirements (Wilson and Poe, 1985; Watanabe and Kiron, 1994). The microencapsulated diet consisted on 70% protein, of which 31.1% was casein (minimum required to obtain this type of capsules) (table 1.) The microencapsulated diet was formulated in order to obtain an AA diet profile as close as possible to the larval carcass profile (see Saavedra et al., 2006). AA in deficiency in the diet were supplemented with crystalline indispensable AA in the balanced diet. The same percentage of free indispensable AA was added to the unbalanced AA diet in the form of crystalline dispensable AA, in order to keep digestible nitrogen comparable in the two diets. Diets were formulated to be isonitrogenous at intake, taking into account the amino acid leaching estimates provided by Yúfera et al. (2002) meaning that to all free AA in the diet a leaching percentages was added (*eg.* if the leaching percentage of a certain free AA is 50% its content in the diet was increased 50%). Microencapsulated diets were prepared according to Yúfera et al. (1999). Fatty acid profiles of the diets are presented in table 2.

Feeding Protocol

Feeding protocol for the control treatment consisted of *Brachionus plicatilis* (5/ ml) enriched with Protein Selco (INVE Aquaculture, Belgium) from 3 to 20 DAH. At day 12, larvae started having *Artemia* nauplii (BE 480, INVE Aquaculture, Belgium) (0.125/ ml) and *Artemia* metanauplii (0.25/ ml), enriched with Rich (Rich®, Greece) from 17 DAH until the end of the experiment. Twice every day *Tetraselmis* spp. and *Isochrysis galbana* was added to the tanks in order to obtain an average concentration of 18 x 10^3 and 82x 10^3 cells/ ml, respectively.

The feeding protocol for balanced and unbalanced diet treatments was the same as the control until the 11 DAH. From 12 to 14 DAH the live food was cut to half of the control and afterwards *Brachionus plicatilis* and *Artemia* were only given at 10% of the control. Microencapsulated diets were introduced at 8 DAH. From 8 to 10 DAH 0.5 g was given to each tank, at 11 and 12 DAH 1 g was given and from 13 to the end of experiment 1.5 g. The micro encapsulated diets were provided to the fish larvae by hand during the day at 10 am, 12 am, 3 pm and 6 pm and in during the night by a automatic feeder at 9 pm, 3 am and 9 am. Before given to the larvae, the micro encapsulated diets were quickly hydrated in 100 ml of fresh water and then spread around the tanks. Being hydrated, the microencapsulated diets remained at the surface for a shorter period and then settled at the bottom. Bottom water was hand flushed three to four times a day. The automatic feeder was equipped with a plastic tube and a water inlet in order for the hydration to occur.

Ammonia excretion

Ammonia excretion was determined using trials of 10 fish larvae enclosed in 45 ml spherical glass vials for two hours. Trials were performed both in fasted larvae (about 12 hours after last meal) and in fed larvae (about 4 hours after feed addition to tanks). The vials were filled with oxygen saturated sea water and sealed without any air bubbles. Two replicates from each tank were used (five replicated *per* treatment) as well as three blanks *per* treatment. Trials were carried out at a temperature of 19 ºC and 38 g/l salinity. When trials were finished, larvae were rinsed in distilled water and frozen for dry weight determination. The sea water was filtered, 20 µl of sulphuric acid 25% was added and then frozen in 20ml plastic flasks for ammonia quantification. Ammonia concentration was determined according to Berthelot (Grasshoff, 1983). Samples were treated with alkaline citrate, sodium hypochloride and fenol in the presence of sodium nitroprussiate which catalyzes the reaction. The blue colour formed by indofenol plus ammonia reaction was measured at 630 nm. Ammonia excretion (expressed as µmol g DW^{-1} h^{-1}) was calculated as follows:

$MNH_4^+ = \Delta[NH_4^+]\ V_{H20}\ DW^{-1} \Delta T^{-1}$, where $\Delta[NH_4^+]$ is the difference between the sample and the blank water, V_{H2O} is the volume of water in the spherical glass, DW is the larvae dry weight, and ΔT is the time duration of the trial.

Deformity analysis

At 15 and 25 DAH, 30 larvae were taken from each tank to observe deformities in the vertebral column. Cartilage was stained with alcian blue (40 minutes) whereas ossified bone was stained with alizarin red (2 hours), according to Gavaia et al. (2000). To determine deformities, Koumoundouros et al. (2001) development descriptors were used as a standard.

Sampling and biochemical analysis

Larvae samples were taken from every tank at 0, 15 and 25 DAH for dry weight determination. Each sample consisted of 40 and 20 larvae at 25 DAH. At 20 DAH, 20 larvae were taken from each tank for fatty acid analysis. These samples were frozen in liquid nitrogen at -190 ºC, then freeze-dried (RVT 400, Savant, NY) and weighed. Ingestion of microencapsulated diet in the larval gut was monitored from 8 DAH to 15 DAH by observation of 30 larvae *per* treatment.

Amino acids analysis

Protein-bound amino acids samples were hydrolysed in 6M HCL at 108ºC over 24h in nitrogen-flushed glass vials. A reversed-phase high pressure liquid chromatography (HPLC) in a Waters Pico-Tag amino acid analysis system, using norleucine as an internal standard, was used. The resulting chromatograms were analysed with Breeze software (Waters, USA).

Fatty acid analysis

After freeze drying, fatty acid composition was determined using the transesterification method by basic catalysis (Park et al., 2001). Fatty acid methyl esters (FAME) were separated and quantified using a Varian CP 3800 gas chromatograph equipped with a flame ionization detector (250 ºC) and a DB-WAX Polyetilene Glicol column (30mx0.25 mm ID.0.25 μm). Injector temperature was maintained constant at 250 ºC over the 40 minutes of the analysis. The column was submitted to a temperature gradient, 5 minutes at 180 ºC, an increase of 4 ºC.min^{-1} for 10 minutes and 220 ºC for 25 minutes.

Data analysis

The relative growth rate (RGR, % DW day $^{-1}$) was calculated using the following formula: RGR= ($e^{(\ln DWt - \ln DW0) / t}$ -1)*100, being DWt and DW_o the final and initial dry weights respectively and t the trial duration. Survival and ammonia excretion data were analysed for significant differences P<0.05 by one-way ANOVA. Post-Hoc analyses were performed using Scheffé test. Growth, fatty acids, larval deformities were analysed by a non-parametric one-way analysis of variance Kruskal-Wallis at a significance level of *P*<0.05.

Results

Amino acid profiles of the diets presented the expected results, a balanced diet with a higher level of indispensable amino acids and an unbalanced diet with higher dispensable amino acid proportion (table 3).

When larva AA profiles are compared to AA profiles of rotifers, balanced and unbalanced microencapsulated diets, a higher similarity is observed between larva and the balanced diet (Fig 1). This is especially evident for arginine, cysteine and valine (Fig 1). The unbalanced diet also seems to have a closer AA composition to the larvae than the control.

Survival rate was significantly higher in the control group ($F_{2,6}$=13.8, p=0.006), almost the double of balanced and unbalanced diet groups (table 4). Growth rate was approximately the same between treatments, both from 8 to 15 days after hatching

(DAH) and from 15 to 25 DAH (table 4). No significant differences were found for larval dry weight between treatments.

Larval guts were observed with a binocular microscope during the first eight days of microencapsulated diet introduction and an increase in the number of larvae with microcapsules in their digestive tract was found (Fig. 2). At the beginning, the percentage of larvae with micro capsules in the gut was approximately 20 % whereas towards the end the proportion increased up to 90% (Fig. 2). It was also possible to observe that most larvae gut ranged from half-full to full and larvae were able to disintegrate the microencapsulated diet.

Significant differences were found for all fatty acids except for total saturated fatty acids (SFA) (table 5). Larvae from control and balanced diets presented significant differences for the arachidonic acid (ARA) ($F_{2,24}$=4.1, p‹0.001). For eicosapentaenoicacid (EPA) as well as docosahexaenoic acid (DHA), larvae presented significant differences between control diet and both balanced and unbalanced diets (($F_{2,24}$=104.6, p‹0.001) and ($F_{2,24}$=127.4, p‹0.001), respectively) (table 5). Larvae from control diet presented higher EPA levels and lower DHA levels when compared with the inert diets. As a result, DHA/EPA ratios of larvae fed balanced and unbalanced diets were three times higher than control.

The ammonia excretion was not significantly different between treatments in fasted larvae (Fig. 3). However, when larvae were fed significant differences were found between control and balanced diet treatments ($F_{2,6}$=9.1, p=0.02). Balanced diet seems to

present almost no difference between fed and fasted larvae and registered the lowest ammonia excretion of all treatments.

At 15 DAH the proportion of deformed larvae was lower than 5%, most of the observed deformities were abnormal shape vertebrae (Fig. 4). At 25 DAH the total frequency of deformed larvae was approximately 40% in the control group, 30% in the unbalanced diet group and 20% on the balanced diet group (Fig. 4). A significant ($F_{2,6}$=0.01, p=0.04) high number of vertebrae fusions were found in the control treatment whereas in the unbalanced diet group the highest frequency of skeletal deformity was vertebral compression although not significantly different. Lordosis was found in control and unbalanced diet groups but none in the balanced diet group (Fig. 4).

Discussion

The substitution of live feed by a compound diet in most marine fish larvae is still difficult and usually performed some weeks after hatching (Cahu and Zambonino Infante, 2001). In this study a casein microencapsulated diet was introduced at 8 DAH and at 15 DAH the live feed was reduced to 10% of the control treatment.

D. sargus larvae acceptance of the microencapsulated diet was quite high achieving almost 100% seven days after its introduction, meaning microencapsulated diet was given in enough quantities and that larvae were able to ingest it. In previous trials, capsules ingestion by larvae was difficult to achieve and only with a pre-hydration of the microencapsulated diet was possible to observe active feeding. The supplementation with free amino acids in the microencapsulated diet was reflected in the HPLC analysis

indicating the expected losses during production and a successful incorporation of these free amino acids.

D. sargus survival rate was higher in the control group which was an expected result as first feeding larvae are commonly dependent on live feed. This difference in the survival rate could be due to an adaptation phase to the microencapsulated diet where mortality rates in the microencapsulated diet treatments were higher. Low success of weaning marine fish larvae in early phases have been reported before (Kolkovski et al., 1997, Cahu and Zambonino Infante, 2001). A lower survival rate could suggest that the 10% of live feed given to the larvae, after the introduction of microencapsulated diet, were not enough to obtain a similar survival rate between dry and live feed treatments. However, survival results from the microencapsulated diet treatment are quite encouraging even if additional work is required until a full weaning is possible in early life stages for this species.

D. sargus larval growth in this study was higher than the reported by Saavedra et al. (2006) for the same period. Larvae from the different treatments presented no significant differences in dry weight which suggests that although live feed promotes a higher survival it does not promote a higher growth rate. In fact, live feed may not be fulfilling larval nutritional needs. Saavedra et al. (2006) reported unbalanced amino acid profiles when rotifers and *Artemia* are used as diet of *D. sargus*. This is consistent with the results obtained in this study for nitrogen excretion. Larvae from the control group, fasted or fed, presented higher ammonia excretion indicating higher nitrogen losses. When fed, the ammonia excretion seemed to increase significantly in both unbalanced

and control treatments. This suggests that *D. sargus* larvae catabolize more protein for energy production after being fed unbalanced dietary AA profiles. When fasted, other nutrients such as lipids might be used in order to spare protein. Still, larvae fed the balanced diet presented lower nitrogen losses, which is in line with previous works (Conceição et al., 2003), and agrees with the higher nitrogen retention for a AA balanced diet observed for *Solea senegalensis* using an in-vivo tube-feeding method (Aragão et al., 2004a). However, the lower nitrogen loss observed in larvae fed the balanced diet did not reflect a higher growth rate. This may be explained by larvae fed the unbalanced diets (microencapsulated and live fed control) compensating for the AA imbalance through a higher feed intake or that AA were used for metabolic processes other than growth.

Fish larvae have a high requirement of AA for energy (e.g., Rønnestad and Conceição, 2005). Therefore, it might be argued that AA imbalances will be negligible in larvae feed a diet containing around 70% crude protein, as happened in the present study. However, this is probably not the case considering the growth rates of up to 30%/day and the low digestive capacity of marine fish larvae (Rønnestad and Conceição, 2005; Tonheim et al. 2005; Conceição et al. 2007). The low capacity to digest proteins and the AA requirements for energy production and growth of marine fish larvae, means AA requirements are likely to be very high and that dietary imbalances will have a burden in terms of nitrogen utilisation (Aragão et al., 2004a) and eventually growth (Conceição et al., 2003).

Concerning fatty acids analysis, a similar pattern between larvae fed a balanced and an unbalanced amino acid microencapsulated diets was observed. This was expected

because both diets had the same fatty acid constituents. For ARA and DHA the microencapsulated diets presented higher larval percentages which were translated into higher DHA/EPA ratios. This ratio is often used to measure eggs and larval quality (Bromage and Roberts, 1995). Larval fatty acid profiles also suggest that the microencapsulated diets used in this study were more efficient in delivering the essential fatty acid to the larvae than enriched rotifers and *Artemia*. A higher level of DHA in the inert diet treatments could also explain the lower proportion of deformed larvae in these groups. Some studies in milkfish evidence that dietary incorporation of DHA induced a decrease of opercular deformities (Gapasin and Duray, 2001). Also, highly unsaturated fatty acids may indirectly regulate some genes involved in skeleton development during ontogenesis (Cahu et al., 2003). Skeletal deformities are one of the constraints to *D. sargus* farming and a frequency of 40% and the control group proves it. Larvae fed the AA balanced diet were almost 50% less affected by deformities than larvae fed live feed only and presented significant lower frequency of vertebral fusions and no case of lordosis. This suggests dietary AA profile might have a role in normal skeleton development.

In conclusion, this study shows that *D. sargus* larvae are still dependent on live feed in order to obtain a high survival rate but when larval quality is concerned, microencapsulated diets seem to promote higher larval quality. Larvae fed rotifers and *Artemia* alone showed very low similarities to the larval AA composition. In contrast, the microencapsulated diets balanced in AA used in this study produced larvae with reduced skeletal deformities, higher DHA levels and lower nitrogen losses.

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Fig 1. Amino acid profiles from 8 DAH larva, control (rotifers), balanced and unbalanced microencapsulated diet. Amino acids are expressed as g. kg^{-1} of indispensable AA (IAA). Larva and rotifers AA profiles from Saavedra et al. (2006).

Fig 2. Percentage of larvae with the microcapsules-filled gut during the first 8 days after the microencapsulate diet was introduced (n=20 larvae *per* tank, three tanks *per* treatment).

Fig. 3. Mass-specific ammonia excretion of fasted and fed 25 DAH *Diplodus sargus* from control, balanced and unbalanced diets. Values are mean and standard deviations

(n=5 of pool of 10 larvae *per* treatment). Different letters represent significant differences for p ‹ 0.05.

Fig. 4. Deformities observed at the dorsal column in *Diplodus sargus* fed on control, a balanced and unbalanced diets (n=60 larvae *per* treatment). AV- Abnormal shape vertebra, HSS- Supranumeric haemal process, VC- Vertebral compression, VF- Vertebral fusion. Different letters represent significant differences for p ‹ 0.05.

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Table 1. Ingredient composition of balanced and unbalanced diets ($g.kg^{-1}$). [1]AgloNorse, Norway [2] Rieber & Son, Norway [3]CPSP 90, Sopropêche (Boulogne sur Mer, France), [4]Acid hydrolysate, Sigma- Aldrich (Germany),[5] Maialab (Portugal), [6] PREMIX (Portugal) [7]Acofarma (Spain), [8]DL- alpha-Tocopherol Acetate Powder, ICN (USA), [9] Rovimix Stay C-35, Roche [*]Sigma-Aldrich, purity ≥ 98%, [+] Fluka (Germany) ≥ 98%.

Ingredients ($g.kg^{-1}$)	BALANCED	UNBALANCED
Fish Meal[1]	159	159
Squid Meal[2]	73	73
Hydrolised Protein Concentrate[3]	73	73
Casein[4]	311	311
Fish Oil[5]	159	159
Vitamin+Mineral Complex[6]	35	35
Agar[7]	05	5
Vitamin E[8]	7	7
Vitamin C[9]	14	14
Threonine[*]	55	-
Arginine[*]	55	-
Phenylalnine[*]	10	-
Cystein[*]	14	-
Lysine[*]	42	-
Serine[+]	-	55
Alanine[+]	-	38
Glutamate[*]	-	38
Proline[*]	-	38

Table 2. Fatty acid profile of *Diplodus sargus* microencapsulated diet. Values are expressed g. $kg^{-1}$of total fatty acids.

Fatty acid	**g. kg^{-1}**
14:0	39.1
16:0	138.3
18:0	29.4
Total – SFA	217.7
16:1	54.0
18:1	187.2
Total - MUFA	415.5
18:2n6	107.6
18:3n3	18.7
20:4n6	4.0
20:5n3	46.9
22:5n3	21.3
22:6n3	75.6
Total – PUFA	342.2
Sn3	190.0
Sn6	148.9
Sn3/Sn6	12.8
n-3 HUFA	143.8
DHA/EPA	16.1
EPA/ARA	117.8

Table 3. Amino acid profiles of balanced and unbalanced *Diplodus sargus* microencapsulated diets. Values are mean and standard deviation and expressed as percentage (g. kg^{-1} of AA).

	BALANCED	UNBALANCED
IAA		
Leu	84.3 ± 3.7	87.0 ± 4.4
Lys	79.9 ± 1.7	75.3 ± 9.8
Arg	74.7 ± 5.9	56.1 ± 1.2
Val	52.3 ± 5.1	55.1 ± 3.9
Thr	56.0 ± 4.4	43.8 ± 2.4
Ile	43.6 ± 5.2	46.3 ± 4.5
His	25.0 ± 1.0	26.6 ± 1.1
Met	24.7 ± 3.1	18.3 ± 5.4
Cys	9.1 ± 1.4	3.0 ± 0.4
Phe	55.2 ± 1.8	48.1 ± 2.4
Tyr	46.2 ± 2.0	48.0 ± 2.7
DAA		
Glu+Gln	161.7 ± 1.0	174.0 ± 7.3
Ala	43.5 ± 1.9	51.5 ± 2.1
Gly	42.3 ± 4.1	39.3 ± 3.2
Asp+Asn	69.0 ± 1.6	70.3 ± 1.5
Ser	52.7 ± 2.3	68.0 ± 6.1
Pro	79.8 ± 0.7	89.1 ± 3.8

Table 4. Growth and survival of *Diplodus sargus* fed with a balanced diet and unbalanced diet. Values are mean and standard deviations (for growth n=4 of 5 larvae pools).

	CONTROL	BALANCED	UNBALANCED
DRY WEIGHT (mg)			
8 DAH	0.04± 0.00	0.05 ± 0.02	0.04 ± 0.02
15 DAH	0.25 ± 0.03	0.28 ± 0.04	0.28 ± 0.05
25 DAH	0.87 ± 0.14	0.78 ± 0.16	0.68 ± 0.07
RGR (%/DAY)			
0-8 DAH	4.3 ± 1.0	8.1 ± 4.1	6.2 ± 5.4
8-15 DAH	32.5 ± 1.6	29.4 ± 7.6	32.2 ± 9.4
15-25 DAH	13.3 ± 0.6	10.8 ± 2.8	9.3 ± 1.1
SURVIVAL (%)	8.6 ± 1.3[a]	4.2 ± 0.6[b]	3.2 ± 1.8[b]

Table 5. Fatty acid profiles of *Diplodus sargus* larvae at 25 DAH fed with a balanced diet, an unbalanced diet and a control diet (n=90 larvae *per* tank, three tanks *per* treatment). Different letters represent significant differences for p ‹ 0.05.

$g.kg^{-1}$	**Unbalanced**	**Balanced**	**Control**
14:0	11.7± 1.6 ^{a}	10.7±1.1^{a}	7.6±0.5^{b}
16:0	152.5±6.3^{a}	150.9±6.8^{a}	142.2±5.3^{b}
18:0	66.1±2.8^{a}	61.3±3.2^{a}	78.5±5.4^{b}
Others	14.7±1.3^{a}	11.4±1.6^{b}	14.1±0.4^{b}
Total - SFA	244.9±9.9	234.3±8.5	242.4±8.7
16:1	30.7±2.7^{a}	26.8±1.4^{b}	27.8±2.2^{b}
18:1	156.0±21.8^{a}	135.7±3.6^{b}	203.0±4.0^{c}
20:1	24.9±4.9^{a}	21.6±1.4^{ab}	21.1±1.5^{b}
Others	21.0±5.3^{a}	16.7±2.4^{ab}	16.1±2.1^{b}
Total - MUFA	232.6±33.2^{a}	200.7±3.4^{b}	268.0±8.9^{c}
18:2n6	58.8±8.2^{a}	71.7±4.0^{b}	76.8±2.2^{b}
18:3n3	31.7±26.0^{a}	12.5±1.4^{a}	109.6±9.4^{b}
18:4n3	7.9±6.1^{a}	2.9±0.4^{a}	20.5±2.7^{b}
20:4n6 - ARA	12.1±5.5^{ab}	15.0±0.8^{a}	10.8±0.8^{b}
20:4n3	8.2±1.2^{a}	6.9±0.8^{a}	14.7± 0.8^{b}
20:5n3 - EPA	68.2±5.8^{a}	66.0±3.8^{a}	96.5±5.2^{b}
22:5n6	4.9±1.2^{a}	5.5±0.5^{a}	3.6±0.6^{b}
22:5n3	26.9±3.4^{a}	29.6±1.7^{a}	15.8±1.4^{b}
22:6n3 - DHA	190.1±31.0^{a}	208.4±7.8^{a}	77.5±6.5^{b}
Others	26.9±7.1	26.3±6.5	29.3±4.2
Total - PUFA	435.7±12.6^{a}	444.9±11.0^{ab}	455.0±3.8^{b}
Sn3	340.1±11.7^{a}	329.9±6.7^{b}	352.3±4.7^{c}
Sn6	95.6±11.4^{a}	115.0±9.1^{b}	102.7±6.0^{a}
Sn3/Sn6	36.1±5.2^{a}	28.9±2.4^{b}	34.4±2.2^{a}
n-3 HUFA	285.2±30.9^{a}	304.0±7.9^{a}	189.8±12.0^{b}
DHA/EPA	28.3±6.3^{a}	31.7±2.5^{a}	8.0±0.5^{b}
EPA/ARA	84.5±73.3	43.9±2.5	90.0±5.4
Non identified	86.9±49.0^{a}	120.2±14.9^{a}	34.7±5.0^{b}

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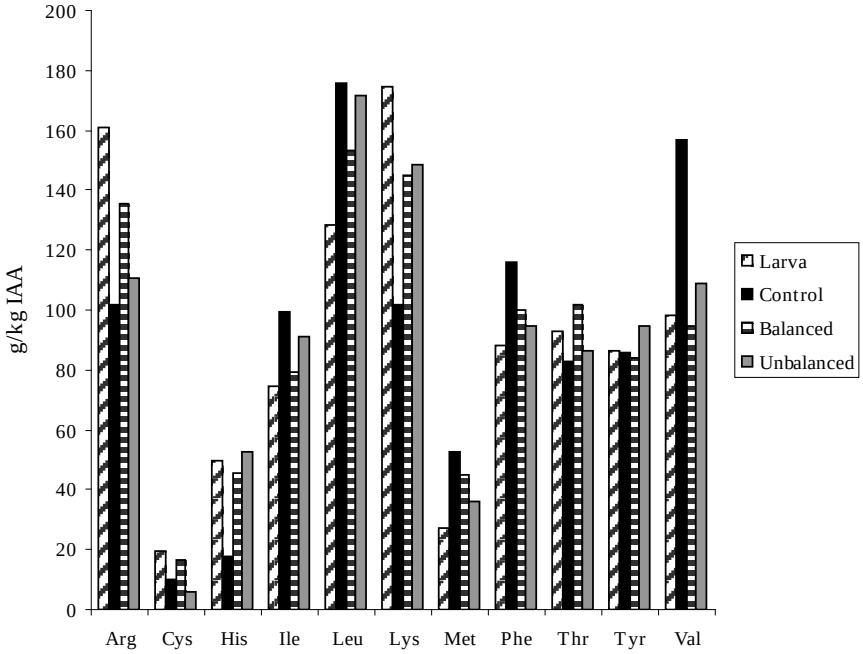
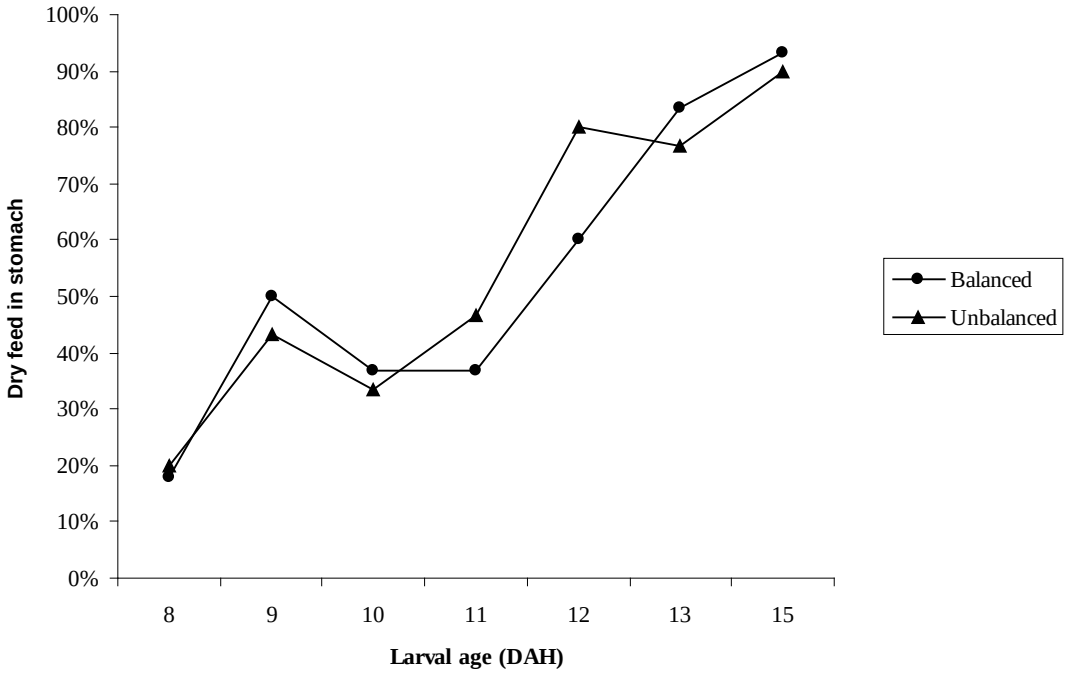
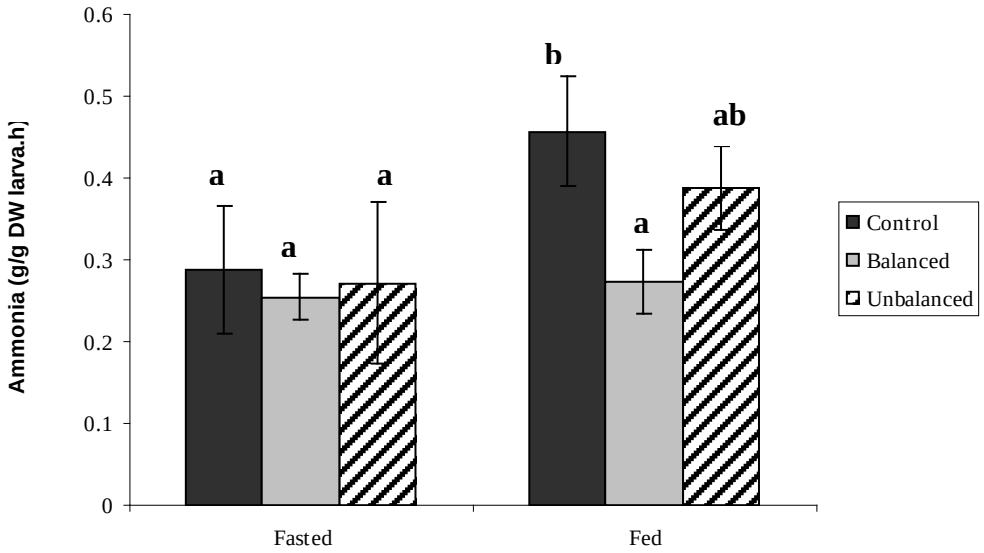
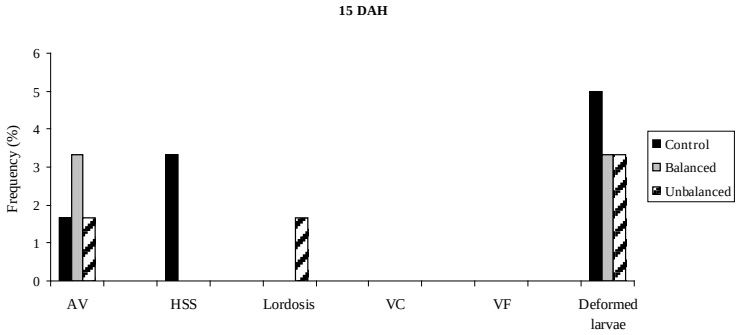
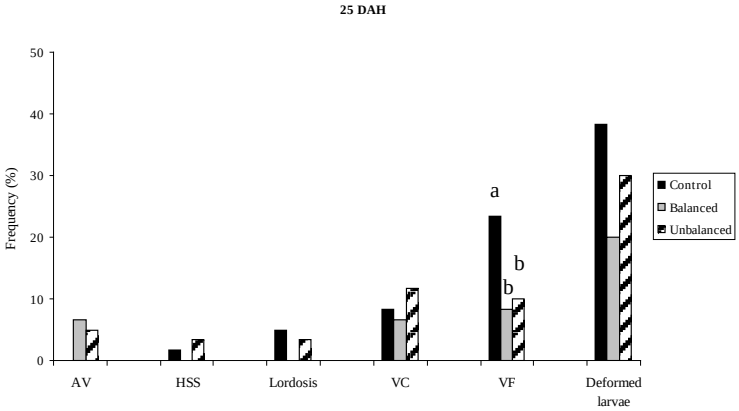



100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Dry feed in stomach
8
9
10
11
12
13
15
Larval age (DAH)
Balanced
Unbalanced


0.6
0.5
0.4
0.3
0.2
0.1
0
Ammonia (g/g DW larva.h)
a
a
a
b
a
ab
Fasted
Fed
Control
Balanced
Unbalanced


15 DAH
Frequency (%)
0
1
2
3
4
5
6
AV
HSS
Lordosis
VC
VF
Deformed larvae
Control
Balanced
Unbalanced

558


25 DAH
Frequency (%)
0
10
20
30
40
50
a
b
b
AV
HSS
Lordosis
VC
VF
Deformed larvae
Control
Balanced
Unbalanced

559