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PLANT-BASED PROTEIN INGREDIENTS CAN SUCCESSFULLY REPLACE FISH MEAL IN THE DIET OF BALLAN WRASSE (*LABRUS BERGYLTA*) JUVENILES

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Abstract

The production of ballan wrasse is hampered by the poor growth and feeding performances and lack of robustness of the species in culture, which is often attributed to sub-optimal nutrition. The commercial marine finfish diets used in ballan wrasse hatcheries, rich in marine ingredients, may not fulfil the species nutritional requirements. This study aimed at evaluating the effects of plant-based ingredients inclusion on the growth, survival, feeding response and digestibility of the species. Simultaneously, the effects of two dietary protein levels were investigated. Ballan wrasse juveniles at approximately 5 g were reared at 15 °C for 70 days. Three Marine Protein:Plant Protein ratios (3.4, 1.6 and 0.9) and two protein contents (51 and 59 % CP) were studied, resulting in five experimental diets and one commercial control diet, tested in triplicate. The partial replacement of fish meal by plant-based ingredients was shown to not compromise growth, survival and feed efficiency. Fish fed the Standard CP diets (51 % CP) showed a significantly lower FCR (1.2 ± 0.1) compared to fish fed High CP diets (59 % CP) (1.4 ± 0.2). Regarding daily feed intake, fish fed the Standard CP diets ate less (1.4 ± 0.2 % day^{-1}) than fish fed the High CP diets (1.6 ± 0.1 % day^{-1}). Signs of enteritis were observed in two out of three High CP diets. The use of plant-based ingredients, more cost-effective and sustainable than fish meal, has a great potential for the species as they reflect better natural feeding of wild populations thus may promote the hatcheries productivity by reducing feed costs while improving their sustainability index. The study shows that a potential route for optimising diet formulation for ballan wrasse may lie within carbohydrate and lipid contents. Overall, this research contributes to the development of ballan wrasse on-growing feeds to promote the development of ballan wrasse aquaculture and its benefits on sea lice mitigation.

Keywords: cleaner fish; ballan wrasse; protein; plant-based diet, digestibility.

1. Introduction

Fish meal has been the predominant protein ingredients used in aquafeeds (Gatlin *et al*., 2007) as is particularly well suited for piscivorous marine finfish species due to its high protein content, amino acid profile and palatability (Rolland *et al*., 2015). However, the global fish meal production is not sustainable as it relies on over-exploited pelagic marine fish stocks (FAO, 2018). Plant-based proteins are an interesting alternative due to their nutritional profile, high availability and price competitivity (Gatlin *et al*., 2007; Hardy, 2010). They have been successfully used to entirely or partially replace fish meal in the formulation of aquafeeds for rainbow trout (*Oncorhynchus mykiss*) (Lazzarotto *et al*., 2018), Atlantic salmon (*Salmo salar*) (Egerton *et al*., 2020; Hartviksen *et al*., 2014; Taylor *et al*., 2019; Vera *et al*., 2020), Atlantic cod (*Gadus morhua*) (Hansen and Hemre, 2013) and European seabass (*Dicentrachus labrax*) (Kaushik *et al*., 2004; Torrecillas *et al*., 2017). However, recent research showed plant-based diets require higher dietary micronutrients to maintain optimal performance in salmon (Vera *et al*., 2020) and nutritional programming can promote better performances (Balasubramanian *et al*., 2016; Clarkson *et al*., 2017). Plant-based diets are of particular interest in the formulation of omnivorous species diets like ballan wrasse (*Labrus bergylta*) as they may better match the species natural food habits, which includes grazing on macroalgae (Sayer *et al*., 1996).

Ballan wrasse became an important cleaner fish species in Northern Europe over the last 10 years for the biological control of sea lice in Atlantic salmon (Brooker *et al*., 2018). While a majority of the deployed wrasse remains wild caught, the sector aspires to be self-sufficient in farmed ballan wrasse in the coming years (Brooker *et al*., 2018). However, the biological knowledge of the species, and its nutritional requirements along the production cycle, remains scarce. Although significant progress has been made in the last decade, the

production is still hampered by slow growth, poor feeding performance, lack of robustness with elevated mortality levels and costly feeds (Cavrois-Rogacki *et al*., 2019).

A very limited number of studies have investigated the protein requirements for the species, with both experimental and commercial CP levels ranging from 36.2 % to 80.9 % in the ongrowing stage (Hamre *et al*., 2013) and 67.4 % to 75.7 % in the weaning stage (Kousoulaki *et al.*, 2015, 2016). Overall, the commercial grower diets for the species, containing around 51 % crude protein (Cavrois-Rogacki *et al*., 2019), have been suggested to be deficient in protein, most likely explaining to a certain extend the slow growth of the species in captivity (Hamre *et al*., 2013). In addition, digestibility of proteins, lipids and energy was shown to be poor in ballan wrasse juveniles compared to that of other marine species (*e.g.* European sea bass, Atlantic cod) (Cavrois-Rogacki *et al*., 2019) and was further supported by reduced feed efficiency and growth. A recent study also suggested that conventional extruded feeds, which are commonly used in ballan wrasse farms, may have a detrimental effect on the apparition of early life stages skeletal deformities as well as severely affect survival and condition during the on-growing phase (Kousoulaki *et al*., 2021).

Ballan wrasse nutritional requirements may be related to its agastric digestive system and absence of acid phase digestion, reducing the digestive capability of the species compared to that of gastric fish (Lie *et al*., 2018). As shown for other stomachless fish species such as common carp (*Cyrpinus carpio*), feed performance is highly dependent on nutrient assimilation from specific raw ingredients (Heinitz *et al*., 2016; Viola *et al*., 1983). The combined use of soy protein concentrate (SPC) and pea protein, which contain around 48 and 22 % crude protein (CP), respectively, are commonly used as an alternative plant-based protein source to marine proteins. In addition, SPC has a good inorganic amino acid (IAA) profile, despite a deficiency in methionine that can be overcome by IAA supplementation (Glencross *et al*., 2007; Rolland *et al*., 2015). Pea protein is interesting due to its high filling

power that can allow the replacement of indigestible starch sources (Collins *et al*., 2013; Guillaume *et al*., 1999; Øverland *et al*., 2009).

The aim of this study was to test the use of SPC and pea protein as a more sustainable substitute to fish meal in ballan wrasse grower diets. Farmed ballan wrasse were fed six experimental diets, with three inclusion levels of plant-ingredients for a duration of 10 weeks. In addition, we investigated the effects of a high dietary protein content (59 % CP) compared to that of a standard protein content (51 % CP).

2. Materials and Methods

2.1. Experimental system and conditions

The trial was carried out at the Marine Environmental Research Laboratory (Machrihanish, UK). The pumped seawater was filtered down to 10 µm through a combination of sand and pressurised filters followed by UV treatment (1.18 mJ h^{-1}). The study was performed in 18 circular flat-bottom tanks (100-l). Each group of 6 tanks had its own recirculation aquaculture system (TMC system 1,000: Tropical Marine Centre, Chorleywood, UK). Water turnover in the tanks was approximately 100% h^{-1}. Lights were on 24 hours with an average intensity of 0.8 W m^{-2} at the water surface. At the beginning of the trial, tanks were stocked with 80 ballan wrasse juveniles (5.0 ± 0.2 g) originating from a commercial ballan wrasse hatchery (Otter Ferry Seafish Ltd, Otter Ferry, UK). Fish were acclimated to the study temperature at a rate of 0.5 ºC day^{-1} and were then maintained at 15.0 ± 0.5 °C during the trial according to Cavrois-Rogacki *et al*. (2019). Water oxygen saturation was kept at 100 % during the trial.

2.2. Feeding and digestibility study

For the first 70 days, termed the “growth trial”, individual tanks were fed one of either the control diet (BioMar Symbio, D1) or five experimental diets (D2-D6) produced by BioMar Ltd. (Brande, Denmark). The diets were hot extruded at >100 °C and air dried for 40-60 mins under hot air (100-125 C) until moisture levels (circa 75 % DW) were reached. Two levels of protein content and three levels of protein source were tested. Diets 1 to 3 corresponded to the standard crude protein diets (“Standard CP”), with a targeted level of 51 % CP, while diets 4 to 6 were the high crude protein diets (“High CP”), with a targeted level of 59 % CP. The protein source was mostly marine in diets 1 and 4 (Marine Protein:Plant Protein ratio “MP:PP” of 4.0 and 2.8, respectively), mixed between plant and marine in diets 2 and 5 (MP:PP of 1.7 and 1.5, respectively) and mostly plant in diets 3 and 6 (MP:PP of 0.9 and 0.8, respectively). In all diets, krill meal level was constant at 30 % (Table 1). The diets were iso-energetic and were tested in triplicate (n = 3, one diet replicate within each recirculation system). Feeds were automatically distributed using Eheim twin-screw feeders controlled by a central command unit, which delivered small amounts of feed every 8 minutes.

Throughout the experiment, all fish were fed over 24 hours to satiation plus excess confirmed by the presence of uneaten pellets at the bottom of the tanks. Feed recovery was done daily from Monday to Friday by siphoning the bottom of the tanks to collect uneaten pellets, which were then weighed and converted into a dry weight using standard curves. During the weekend, fish were fed based on the average of the weekly feed intake and an average of the previous feed waste was used. At the end of the growth trial, the experiment was extended for another 2 weeks to collect faecal material for digestibility analyses. Yttrium oxide, an inert digestibility marker, was added to the diets during the production process at 400 mg kg^{-1}. Fish were fed continuously to satiation for 2 days before turning off the feeders and flushing the tanks to remove the faeces and uneaten food. On the 3rd day, the freshly produced faeces were collected by siphoning every 2 hours, and storing samples at -20 ºC. The procedure was

repeated until enough faecal material (*circa* 14 g wet weight per tank) was collected for nutritional analysis.

2.3. Sampling

The sampling regime during the growth trial included an initial (D0) and final (D70) sample point for which the total biomass (g), the number of fish per tank and the individual total length (cm) and weight (g) (n = 20 per tank) were recorded. Tanks were checked daily for mortalities and calculations were adjusted accordingly. Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Daily Feed Intake (DFI) and condition factor (K) were calculated as follow:

$$FCR = (\text{feed intake, g}) / (\text{weight gain, g})$$

$$SGR\ (\%\ \text{biomass day}^{-1}) = 100 \times (\ln(\text{final body weight, g}) - \ln(\text{initial body weight, g})) / (\text{time, days})$$

$$DFI\ (\%\ \text{biomass day}^{-1}) = FCR \times SGR \text{ (Laird \& Needham, 1988)}$$

$$K = 100 \times (\text{weight, g}) / (\text{length, cm})^3$$

At the end of the growth trial, three pools of four fish per tank were sampled and individually analysed to account for variability within a tank. The resulting data was averaged and the average of each experimental triplicate (i.e. tank) were used for the subsequent statistical analysis.

The foregut (first quarter of the intestinal tract) of five sacrificed fish per tank was dissected and stored in pure ethanol at room temperature for histological analysis. All carcasses were stored at -20 °C until later proximate composition analysis.

2.4. Proximate composition

Proximate composition of the feeds and fish carcasses were determined according to standard procedures (AOAC, 2000) in place at the Nutrition Analytical Service laboratory of the Institute of Aquaculture (Stirling, UK). All samples were analysed in technical duplicates (n = 2). Before analysis, feed samples were ground with a mortar and pestle while the carcasses were homogenised in a blender to produce a paste. Moisture content was calculated after drying weighed samples in an oven at 110 °C for 24 h and ash content determined after incineration of a weighed sample at 600 °C for 16 h. Crude protein content was measured by determining N content (N × 6.25) using automated Kjeldahl analysis (KjelROC analyser; OPSIS). Energy content was measured using bomb calorimetry calibrated with benzoic acid (Gallenkamp Autobomb; Gallenkamp & Co. Ltd). Crude fibre in the diets was measured after de-fattening the samples in petroleum ether and digestion in 1.25 % sodium hydroxide followed by a digestion in 1.25 % sulphuric acid (Fibercap system, Foss). Crude lipid content was measured by extraction of the total lipids by homogenisation in chloroform/methanol (2/1, v/v) according to Folch *et al*. (1957). Fatty acid methyl esters (FAME) analyses of diets were prepared according to Christie (2003) from total lipids by acid-catalysed transesterification at 50 °C for 16 h. FAME were separated and quantified by GLC using a Fisons GC-8160 (Thermo Scientific) equipped with a 30 m × 0.32 mm internal diameter × 0.25 μm ZB-wax column (Phenomenex), on-column injector and a flame ionisation detector. Data were collected and processed using Chromcard for Windows (version 2.01; Thermoquest Italia S.p.A.).

Faeces collected during the digestibility study were freeze-dried for 72 hours and homogenised. Proximate composition was assessed similarly to that of the diets and carcasses. To determine their yttrium oxide content, diets and faeces were digested in 65 % nitric acid in a microwave (MARSXpress, CEM) for 40 min (20 min ramping to 120 ºC and

20 min holding that temperature). Digests were transferred into a volumetric flask and made up into x 25 dilutions with distilled water. Samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Scientific Model X Series 2, US). Apparent digestibility coefficient (ADC) was calculated as follow:

$ADC_{nutrient}$ (%) = [1 – ($Nutrient_{faeces}$, %) / ($Nutrient_{diet}$, %) x ($Yttrium_{diet}$, mg kg^{-1}) / ($Yttrium_{faeces}$, mg kg^{-1})] x 100

2.5. Proximal intestine histology

The first quarter of the intestine (*i.e.* proximal intestine) was dissected and stored in 10 % buffered formalin. Samples were processed according to standard histological methods in place at the Institute of Aquaculture (Stirling, UK). Briefly, the samples were dehydrated in ethanol, equilibrated in xylene and embedded in paraffin. Transverse cuts of approximately 5 µm were stained with haematoxylin and eosin. Each slide was observed under microscope and intestine subjectively scored to quantify severity of potential enteritis according to Urán *et al*. (2008) with overall enteritis score (Table 2; Fig. 1).

2.6. Statistical analysis

All data are presented as means ± standard deviations. Percentage data were subjected to arcsine square-root transformation prior to statistical analyses. Normality and homogeneity of variance in the data were confirmed using Shapiro-Wilk and Levene's tests, respectively. Data were analysed by two-way ANOVA, with protein content and protein source as factors, followed by Tukey's post-hoc test when relevant ($P < 0.05$). Data for SGR and DFI were not normally distributed and could not be normalised through data transformation. They were therefore analysed using the Kruskal-Wallis non-parametric test ($P < 0.05$). Digestibility data

are expressed as one value per diet as the faeces for each triplicated treatment were pooled and therefore no statistical analysis could be performed. All data were analysed using SPSS (IBM SPSS Statistics 23, NY, US) and Microsoft Excel (v16, WA, US).

3. Results

3.1. Diet proximate composition

Diet moisture levels ranged between 10.6 ± 0.1 % (D5) and 12.3 ± 0.1 % (D3 and D6). The Standard CP diets displayed protein levels ranging from 48.5 ± 0.5 % (D2) to 51.7 ± 0.2 % (D1) and the High CP diets displayed protein levels ranging from 55.8 ± 0.4 % (D6) to 59.4 ± 0.3 % (D4) (Table 1). Lipid levels were on average 11.6 ± 0.4 % in the High CP diets and 10.6 ± 0.5 % in the Standard CP diets. Fibres levels increased as the inclusion of plant material increased, with 15 % and 67 % more fibre in the plant source diets compared to that of the 50/50 diets and marine diets, respectively. The Carbohydrate/Lipid ratio (CHO:L) was about 50 % higher in the Standard CP diets compared to that of the High CP diets. In terms of fatty acids, the Total n-6 levels increased by up to 56 % with the inclusion of plant ingredients, mostly due to the linoleic acid (18:2n-6). The total n-3 levels were up to 22 % higher in the marine based diets, mostly due to the levels of DHA (22:6n-3).

3.2. Growth performance

No differences between treatments were observed in total length, weight, K, SGR and mortality at the end of the 10-weeks growth period (Table 3). Overall, the fish grew by 150 % compared to their initial weight. K factor was at 1.6 ± 0.0 at the beginning of the trial and ended at 1.7 ± 0.0. SGR was comparable in all diets averaging 1.2 ± 0.1 %. Cumulative mortality of the stock as a whole was 3.5 % by the end of the trial. FCR and DFI were both affected by the protein content in the feed but not by the protein source (no interaction) (Fig. 2). The fish fed the Standard CP diets showed a significantly lower FCR (1.2 ± 0.1) compared to that of the fish fed the High CP diets (1.4 ± 0.2). Regarding DFI, the fish fed the Standard CP diets ate less (1.4 ± 0.2 % day^{-1}) than the fish fed the High CP diets (1.6 ± 0.1 %

day^{-1}) with no influence in relation to the protein source. Few unexpected morts were found in two tanks during the experiment, which resulted in a higher (but not significantly) mortality for fish fed D3 and D4. The veterinarian analysis concluded that those morts were most likely due to an episode of amoebic gill disease (AGD) but that the vast majority of the fish stock was AGD free.

3.3. Fish proximate composition

Moisture content of the whole body was significantly affected by dietary protein content and source, but a statistical interaction was observed (Table 4). Overall, moisture was less than 1 % higher in the fish fed High CP and plant source diets. Ash and crude protein content were significantly affected by either protein content or source, however there was no significant interaction between the two factors. Ash content was 4 % higher in the fish fed Standard CP diets (15.0 ± 1.2 %). Also, ash content was 13 and 7 % higher in plant source diets (15.7 ± 0.8 %) compared to that of marine (13.9 ± 0.1 %) and mix source diets (14.6 ± 0.3 %), respectively. Protein content in the whole body was 1.4 % higher in fish fed High CP diets and 2 % higher in fish fed marine or plant diets compared to the mixed source diet. Crude lipid was affected by the protein source and an interaction between protein content and source was found. Fish fed the plant source diets showed whole-body lipid content 23 % lower (9.4 ± 0.5 %) than in fish fed the marine or mixed source diets (11.6 ± 0.9 %). Carbohydrate content was not affected by either dietary parameters or was there a significant interaction of the factors.

3.4. Digestibility

Apparent digestibility coefficients across treatments for proteins, lipids and energy were 87.0 ± 2.2, 78.3 ± 2.9 and 80.4 ± 2.9 %, respectively (Table 4). $ADC_{Proteins}$ were similar across diets however statistical analysis could not be done on the digestibility data, ADC_{Lipids} and ADC_{Energy} were apparently reduced (-6.7 % and -4.6 %, respectively) in the fish fed the plant source diets (*i.e.* D3 and D6).

3.5. Histology

The histological analysis of the intestinal sections showed that there was a significantly higher number of mucus cells in the two of the High CP diets (*i.e.* D4 and D6). The histopathological assessment diagnosed signs of mild intestinal inflammation for the fish fed those diets (Table 5).

4. Discussion

Two areas have been highlighted as priorities in the development of suitable ballan wrasse diets. The first one concerns the source and quality of the raw materials used in the formulation of the diets. The first published data on the digestibility of a commercial ballan wrasse on-growing diet showed that the apparent digestibility coefficients were greatly lower for proteins, lipids and energy than expected for marine finfish (Cavrois-Rogacki *et al*., 2019). Other publications have shown the benefits of using high-quality ingredients on the survival and growth of the species compared to the commonly used standard fish meal (Bogevik *et al*., 2016; Kousoulaki *et al*., 2015, 2021). The second area concerns the dietary protein content, as it has been suggested that ballan wrasse may need high protein diets in order to maximise growth (Hamre *et al*., 2013; Cavrois-Rogacki *et al*., 2019). Based on these earlier findings, the present experiment investigated those two areas by testing standard and high dietary protein contents as well as the protein ingredient's source on the survival, growth and feed efficiency of ballan wrasse juveniles.

In terms of the key performance indicators (KPIs), no significant effects of the diet formulation were shown on growth, condition and survival across diets. Irrespective of the diet offered, fish more than doubled their weight over the 10-weeks growth trial. Specific growth rates of 1.2 % day^{-1} were recorded, which exceeded those observed in previous studies at similar rearing temperature (SGR of 0.8 - 0.93 % day^{-1}, Cavrois-Rogacki *et al*., 2019; Kousoulaki *et al*., 2021). This difference could be explained by two main reasons. First, fish stock and broodstock origin were different in the three studies hence a different genetic potential. Second, the fish in our study were stocked at around 5 g while fish in Cavrois-Rogacki *et al*. (2019) and Kousoulaki *et al*. (2021) were stocked at around 14.5 and 11.4 g, respectively, hence about two to three-fold larger. Fish growth studies have shown that at the same temperature, SGR is at its maximum during the early stages and declines

thereafter (Brett and Groves, 1979; Jobling, 1996). While unexpected mortalities were reported in some tanks, the subsequent veterinarian analysis concluded to a punctual mortality event due to AGD, not correlated to the experiment diets.

Interestingly, this study contradicts the findings of Kousoulaki *et al*. (2021), in which extruded diets were associated to a higher degree of mortality and overall poorer performance compared to agglomerated or cold extruded diets. The mortality rate of the farmed wrasse in Kousoulaki *et al*. (2021) over an 18-weeks growth period ranged between 37.3 and 57.3 % whereas in our study, the mortality ranged between 2.1 and 5 %, yet over a 10-weeks growth period. The condition factor of the fish at the end of the trial in Kousoulaki *et al*. (2021) were about 80 % higher (3.06-3.11) compared to those in our study (1.7-1.8) where the condition factors were much similar to that of wild ballan wrasse (Cavrois-Rogacki *et al*., 2021). Overall, this study is a reflection of the UK's commercial ballan wrasse production figures and may highlight a difference in the KPI for the culture of the species between UK and Norway (Brooker *et al*., 2018; Cavrois-Rogacki *et al*., 2019; Cavrois-Rogacki *et al*., 2021). This study showed that extruded diets and the inclusion of plant ingredients during the on-growing phase of ballan wrasse can maximise growth while preserving the condition of the fish, with very low mortality rates.

In terms of feed efficiency, protein source had no apparent impact. However, fish fed the Standard CP diets had significantly lower FCR and DFI. The FCRs observed in this study are in line with those found by Cavrois-Rogacki *et al*. (2019), where FCRs ranged between 1.4 ± 0.1 and 1.8 ± 0.3 in fish reared at 16 ºC and fed Otohime S2 and BioMar Symbio, respectively. Arguably, FCRs in our study were improved compared to those in Cavrois-Rogacki *et al*. (2019), as FCRs as low as 1.0 ± 0.1 (D3) and averaging 1.3 ± 0.2 were obtained. The improvement is even more striking when compared to previous work carried on ballan wrasse juveniles, where FCRs ranged from 2.07 ± 0.93 to 15.05 ± 24.3 when fed

328 experimental diets and reared at 12 ºC (Chalaris, 2018). This data shows the significant
329 improvements that have been made on feed optimisation for ballan wrasse grower diets over
330 the last years. Nevertheless, these results are also in contradiction with those found in other
331 studies, which suggested that ballan wrasse may require a protein rich diet (*i.e.* >60 % CP)
332 (Hamre *et al*., 2013; Cavrois-Rogacki *et al*., 2019). For instance, we input our diet
333 macronutrient levels in the growth fitted model presented in Hamre *et al*. (2013), which
334 aimed at establishing the formulation that maximises growth on the species based on the
335 dietary content in proteins, lipids and carbohydrates. The outcomes indicated that our "best
336 formulation" resulted in a weight 25 % smaller (3.73 g) than the highest weight obtained in
337 Hamre *et al*. (2013) (4.71 g), in which a diet containing 64.6 % CP was used. Interestingly,
338 the high-protein diet rich in marine ingredient (D4) resulted in the highest output using the
339 model, although it did not translate *in vivo*. It would be interesting to see how the 64.6 % CP
340 diet performed in terms of feed efficiency (no data on feeding efficiency was published).
341 Protein rich diets often lead to increased levels of nitrogen discharge with the subsequent
342 negative effects on water quality, particularly in recirculated aquaculture systems, and these
343 should therefore be kept in mind (Rolland *et al*., 2015). Finally, Hamre et al. (2013) diet was
344 formulated with 53.7 g 100 g^{-1} DW of cod filet. Cod filet is a very expensive product
345 compared to plant-based protein and therefore a full cost-benefit analysis would be required
346 to evaluate the potential of this ingredient in the formulation of on-growing diets for ballan
347 wrasse.

348 In our study, the High CP diets did not perform as well as the Standard CP diets, with higher
349 FCRs and DFIs. Although there is no published data on the FCR and DFI of ballan wrasse
350 fed protein-rich diets that would allow the comparison, Hamre *et al*. (2013) suggested that the
351 feed intake was probably higher in fish fed high protein diets (*i.e.* >60 % CP) based on a
352 condition factor analysis, similarly to our findings. The apparent digestibility coefficients for

proteins, lipids and energy were in line with other studies on marine finfish (Mundheim *et al*., 2004; Glencross *et al*., 2007) and were greatly higher than those obtained by Cavrois-Rogacki *et al*. (2019). The reasons for such differences between studies remain unclear. The same faeces collection method was used, including the time period over which the faeces were sampled (*i.e.* between 2 and 3 weeks). However, the ADCs in Cavrois-Rogacki *et al*. (2019) were calculated by averaging the values of three tanks whereas in our study, the ADCs were calculated based on a technical duplicate of pooled faeces from three tanks, due to a limiting amount of faecal material. Ideally, this highlights the need for a dedicated digestibility study on ballan wrasse. Despite the lack of statistical analysis, some trends could be observed. Proteins seemed to be digested at the same level across diets while lipids in fish fed D3 and D6 were the less digested, resulting into a lower body lipid content.

In terms of whole-body macronutrient composition, wild fish are often used as a reference on the assumption that their nutrient requirements are covered by their natural diet. In our study, fish had a similar protein content to that of the wild fish analysed in Hamre *et al*. (2013). Fish fed the High CP diets had a higher protein uptake, which did not translate into better growth, as mentioned earlier. The crude lipid level of the whole bodies decreased with the inclusion of plant ingredients and higher dietary protein, with only fish fed D1 and D2 showing a lipid content similar to that of the wild fish. However, the validity of using wild fish as a proxy for nutrient profiling can be argued. The wild fish in Hamre *et al*. (2013) study were caught during summer 2011, at a period when sea water temperature promotes feeding activity of species like ballan wrasse. The activity of ballan wrasse in the sea cages during winter is known to reduce drastically (Brooker *et al*., 2018; Leclercq *et al*., 2018). During those winter months (*i.e.* from November to March), the gut fulness of related species, goldsinny wrasse (*Ctenolabrus rupestris*) and corkwing wrasse (*Crenilabrus melops*), appeared to be lower according to Sayer *et al*. (1996). This evidence indicates potential dietary changes throughout

the year that could result in a different macronutrient composition of the whole body. For instance, the lipid content of ballan wrasse juvenile farmed at 10 ºC (*i.e.* winter months) was significantly lower than those farmed at temperatures above 13 ºC (*i.e.* summer months) (Cavrois-Rogacki *et al*., 2019). Therefore, the optimal nutrient composition of on-growing diets for ballan wrasse may vary depending on water temperature.

SPC and pea protein are high-quality protein ingredients that have been extensively investigated for their use in aquafeeds as a replacement to fish meal (Carter and Hauler, 2000; Draganovic *et al*., 2011; Davidson *et al*., 2016). In species like Atlantic salmon, rainbow trout, European sea bass and gilthead seabream (*Sparus aurata*), plant-based ingredients have successfully been used to partially or totally replace fish meal (Kaushik *et al*., 1995, 2004; Hartviksen *et al*., 2014; Vera *et al*., 2020). Plus, they are also both economically very competitive (Sørensen *et al*., 2011). With SPC and pea protein costing £700-800 and £1,100-1,300 per ton, respectively, they are cheaper than FM (*i.e.* £1,300-1,500 per ton). A gross cost-analysis, looking into the cost of the block made of FM-SPC-pea protein, indicates that for D1 (*i.e.* no SPC or pea protein, only FM), FM accounts for £1,400 per ton of diet. In D2, the substitution of FM by SPC and pea protein drops the price of the block by 18 % to £1,145 per ton and in diet 3 by 36 % to £897 per ton. However, plant raw materials have two disadvantages, the first one being the presence of antinutritional factors (ANF) such as glucosinolates, saponins or tannins (Francis *et al*., 2001). Some ANF can be eliminated by elevated heat treatment (Francis *et al*., 2001; Drew *et al*., 2007), which was the case in our study since the diets had been hot extruded at more than 100 °C. The second disadvantage is the lack of some essential amino-acids (AA) that needs to be compensated by the inclusion of supplementary inorganic amino-acids (IAA) (Rolland, 2015). Although we did not analyse the dietary AA levels, the diets were formulated using krill meal (30 g 100 g diet^{-1}) and fish meal (8-32 g 100 g diet^{-1}), both rich in essential AA (Refstie *et al*., 2004; Rolland *et al*.,

403 2015). In addition, the diets were formulated so that minimum requirements for marine
404 finfish species were met, based on NRC recommendations (2011) for marine species sharing
405 similarities with ballan wrasse in terms of feeding habits, ecology and physiology such as sea
406 bass (*Dicentrachus labrax*) and Atlantic cod (*Gadhus morua*). One challenging point in the
407 formulation of the experimental diets was the starch levels. Indeed, starch contents were 2.5
408 times lower in the High CP diets compared to the Standard CP diets. Starch level can
409 considerably affect the digestibility of aquafeeds, with optimal levels ranging from 15 to 30 g
410 100 g^{-1} (Glencross, 2006). However, the apparent digestibility coefficients for protein and
411 energy were comparable across diets, despite the higher starch content of the Standard CP
412 diets. Starch is used as a filler and therefore, the High CP diets, richer in protein ingredients,
413 needed less starch compared to the Standard CP diets. As a consequence, the carbohydrate
414 levels were lower in the High CP diets. A CHO:L ratio above 2 was shown to reduce growth
415 performance in other teleost species (Ali and Jauncey, 2004; Hu *et al*., 2007). However, since
416 we know that the CHO:L ratio is species and temperature specific, further investigation is
417 needed in ballan wrasse.

418 Polyunsaturated fatty acids (PUFA) from the omega 6 and 3, particularly arachidonic acid
419 (ARA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA,
420 22:6:n-3), are considered to be essential to marine finfish at all life stages (Sargent *et al*.,
421 1999). ARA is a precursor of 3-series antagonistic prostaglandins that provide a low-
422 inflammatory response (Tocher, 2003) as well as playing a major role in oogenesis at adult
423 stage (Grant *et al*., 2016). EPA and DHA, although of known importance during early stages
424 of development, are critical for the normal cellular function of all fish species (Tocher, 2015;
425 Tocher *et al*., 2010). Unlike marine ingredients, plant ingredients completely lack long-chain
426 (≥ C20) PUFA (LC-PUFA) including the above mentioned essential fatty acids (EFA). The
427 inclusion of plant ingredients in our study clearly showed the decreased accumulation of LC-

PUFA, and DHA in particular. Although the specific requirement in EFA for ballan wrasse remain unknown (Kabeya *et al*., 2018), feed formulators should remain vigilant regarding the inclusion of plant-ingredients in the diet of ballan wrasse, so its minimal requirements are not compromised and remain within recommendations for marine species such as Atlantic cod or European seabass (NRC, 2011) until further data is collected on the species nutritional needs.

The histopathological assessment of the proximal intestine region revealed a mild inflammation in the fish fed D4 and D6 (High CP), characterised by an overall higher cellularisation (*i.e.* increased number of mucus cells). It was suspected that the inclusion of plant raw materials, and particularly soy-based ingredients, may induce higher cellularisation and mild enteritis as previously shown in other finfish species (Urán *et al*., 2008, 2009; Merrifield *et al*., 2011; Gu *et al*., 2016). It remains unclear why D5 showed the lowest score at the histological assessment in the High CP diets, comparable to that of the control diet D1. It is also interesting since D5 showed the highest digestibility coefficients, which could have been promoted due to a lesser degree of enteritis compared to D4 and D6. Further nutritional analysis of the feeds, such as minerals, would help identify potential differences that could explain this result. The data indicates that the inclusion of plant ingredients should be done with cautious as to not induce intestinal inflammation, which can result in lower nutrient digestibility and poorer feed efficiency (Knudsen *et al*., 2008; Urán *et al*., 2009).

In conclusion, the study showed that the partial replacement of FM by plant-based ingredients, namely SPC and pea protein, in the formulation of diets for ballan wrasse juveniles do not compromise growth and feed efficiency. The use of plant-based ingredients, cost-effective and more sustainable than fish meal, has a great potential to promote the hatcheries productivity by reducing feed costs, which often represent more than 50 % of the retail price of the fish. Moreover, the study showed that diets with standard marine finfish protein levels meets the species requirements and even appeared to outperform protein-rich

diets, which may be closer to the species' natural diet and therefore requirements. Signs of enteritis were observed in two of the High CP diets which could impact on gut nutrient absorption and therefore should be carefully considered when formulating protein rich diets for the species. Importantly, results did not suggest any negative impact of extruded diets as recently suggested (Kousoulaki *et al*., 2021) with KPIs among the best recorded so far in the cultured species. Overall, this research contributes to the up-scaling of ballan wrasse production in the global health challenge that the sea lice represents.

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Tables

Table 1. Formulation, proximate composition and selected fatty acids levels of the experimental diets. Proximate composition and fatty acids data represent means ± standard deviation (technical duplicate). DW: dry weight; FA: fatty acid; ND: not detected.

Diet	D1	D2	D3	D4	D5	D6
Protein content targets (% CP)	51			59		
Protein source	Marine	Mixed	Plant	Marine	Mixed	Plant
Ingredients (g.100 g diet^{-})						
Krill meal	30	30	30	30	30	30
Fish Meal	26.5	14.5	2.5	32	20	8
Soy protein concentrate	0	10	14	0	10	8
Wheat Gluten	14	11	14	14	16	18
Pea Protein	0	4.5	10	8	8	20
Starch Source	22	22.5	22	8.5	8.5	8.5
Vitamin and mineral premix[1]	7.5	7.5	7.5	7.5	7.5	7.5
MP:PP[2]	4.0	1.7	0.9	2.8	1.5	0.8
Proximate composition						
Moisture	11.6 ± 0.2	11.3 ± 0.0	12.3 ± 0.1	11.1 ± 0.0	10.6 ± 0.1	12.3 ± 0.1
Ash	8.6 ± 0.1	7.9 ± 0.5	7.3 ± 0.2	8.7 ± 0.1	7.8 ± 0.2	7.2 ± 0.1
Crude protein	51.7 ± 0.2	48.5 ± 0.5	49.6 ± 0.6	59.4 ± 0.3	57.8 ± 0.5	55.8 ± 0.4
Crude lipid	10.9 ± 0.4	10.9 ± 0.5	10.1 ± 0.2	12.0 ± 0.3	11.7 ± 0.3	11.1 ± 0.2
Crude fibre	1.4 ± 0.1	2.2 ± 0.1	2.5 ± 0.0	1.3 ± 0.2	1.8 ± 0.1	2.1 ± 0.2
Carbohydrate[3]	28.8 ± 0.4	32.7 ± 0.6	33.0 ± 0.6	20.0 ± 0.2	22.7 ± 0.8	25.8 ± 0.2
CHO:L[4]	2.7 ± 0.1	3.0 ± 0.2	3.3 ± 0.1	1.7 ± 0.1	1.9 ± 0.1	2.3 ± 0.0
Gross energy (mJ kg^{-1})	18.7 ± 0.1	19.4 ± 0.2	18.3 ± 0.7	19.9 ± 0.2	19.6 ± 0.1	19.3 ± 0.1
Selected fatty acids (% of total FA)						
14:0	8.3 ± 0.1	9.0 ± 0.0	8.6 ± 0.1	7.3 ± 0.1	8 ± 0.0	8.0 ± 0.1
15:0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
16:0	21.6 ± 0.1	21.8 ± 0.1	21.3 ± 0.1	21.2 ± 0.1	21.5 ± 0.0	21.2 ± 0.0
18:0	2.1 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	2.2 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
Saturates	33.0 ± 0.1	33.6 ± 0.2	32.7 ± 0.4	31.6 ± 0.1	32.3 ± 0.0	31.8 ± 0.1
16:1n-9	ND	ND	7.2 ± 0.0	6.8 ± 0.0	7.0 ± 0.0	ND
16:1n-7	7.4 ± 0.1	7.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	6.8 ± 0.0
18:1n-9	15.0 ± 0.1	15.2 ± 0.0	16.0 ± 0.0	16.2 ± 0.0	16.4 ± 0.1	16.6 ± 0.0
18:1n-7	5.0 ± 0.0	5.2 ± 0.0	5.2 ± 0.0	4.6 ± 0.0	5.0 ± 0.0	4.9 ± 0.0
20:1n-11	1.6 ± 0.2	1.3 ± 0.0	1.1 ± 0.0	1.8 ± 0.0	1.4 ± 0.0	1.2 ± 0.0
22:1n-11	1.5 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	1.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0
Monounsaturates	30.9 ± 0.1	30.4 ± 0.1	30.8 ± 0.0	31.8 ± 0.1	31.1 ± 0.2	30.6 ± 0.0
18:2n-6	8.3 ± 0.0	10.2 ± 0.0	14.2 ± 0.0	8.9 ± 0.0	11.0 ± 0.0	14.6 ± 0.0
18:3n-6	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
20:4n-6	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.3 ± 0.0
22:5n-6	0.1 ± 0.0	0.1 ± 0.0	ND	0.1 ± 0.0	ND	ND
Total n-6	9.4 ± 0.0	11.1 ± 0.0	14.9 ± 0.0	10.0 ± 0.0	11.8 ± 0.0	15.4 ± 0.0
18:3n-3	1.5 ± 0.0	1.8 ± 0.0	2.2 ± 0.0	1.9 ± 0.0	2.0 ± 0.0	2.4 ± 0.0
18:4n-3	2.5 ± 0.0	2.6 ± 0.0	2.5 ± 0.0	2.4 ± 0.0	2.5 ± 0.0	2.4 ± 0.0
20:4n-3	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
20:5n-3	10.5 ± 0.0	10.3 ± 0.0	9.3 ± 0.0	9.8 ± 0.0	9.7 ± 0.1	9.2 ± 0.0
22:5n-3	0.6 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
22:6n-3	10.1 ± 0.0	8.4 ± 0.1	5.9 ± 0.0	10.5 ± 0.0	8.8 ± 0.2	6.7 ± 0.0
Total n-3	25.7 ± 0.0	23.9 ± 0.1	20.6 ± 0.1	25.5 ± 0.2	23.9 ± 0.2	21.4 ± 0.0

[1]Vitamin and mineral premix (BioMar Ltd.), [2]Marine Protein :Plant Protein ratio, [3]Carbohydrate = 100 – Ash - Crude protein - Crude lipid, [4]CHO:L: carbohydrate:lipid ratio.

654 **Table 2.** Histological scoring system of morphological changes used to characterise changes

655 induced by enteritis (from Urán *et al*., 2008).

Score	Parameter
Lamina propria (LP) of simple folds	
1	Normal size LP
2	Increased size LP
3	Medium size LP
4	Large size LP
5	Largest size LP
Mucus cells (MC)	
1	Scattered MC
2	Increased number and sparsely distributed MC
3	Diffused number widely spread MC
4	Densely grouped MC
5	Highly abundant and tightly-packed MC
Connective tissue (CT)	
1	Very little CT between base of folds and circular muscle
2	Slightly increased amount of CT beneath some of the MF
3	Clear increase of CT below all MF
4	Thick layer of CT beneath high percentage of MF
5	Extremely thick layer of CT beneath some MF
Mucosal folds (MF)	
1	Simple and complex MF appear long and thin
2	Simple MF have medium length, while the complex MF appear thicker
3	Simple MF had short to medium length, while complex MF are stubby
4	Simple MF are thick and short, while thick and stubby complex MF are prevalent
5	Both complex and simple MF appear very short and stubby
Overall Scoring	
1-2	Normal morphology
3	Clear signs of inflammation
4-5	Chronic symptoms of enteritis

Table 3. Growth performance indicators and mortality in farmed ballan wrasse juveniles reared for 70 days and fed the control (D1) and experimental (D2 to D6) diets. Data are expressed as mean ± SD (n = 3). Data for length, weight, K, FCR and mortality were analysed by two-way ANOVA (2 protein contents × 3 protein sources; * $P < 0.05$; ** $P < 0.01$). Data for Specific growth rate (SGR) and daily feed intake (DFI) were analysed using the non-parametric tests Kruskal-Wallis (* $P < 0.05$; ** $P < 0.01$).

Diet	D1	D2	D3	D4	D5	D6	Significance		
Protein content (% CP)		51			59		Protein content	Protein source	Interaction
Protein source	Marine	Mixed	Plant	Marine	Mixed	Plant			
Initial Length (cm)	6.7 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.0	6.8 ± 0.1	6.7 ± 0.1	ns	ns	ns
Final Length (cm)	9.0 ± 0.1	9.1 ± 0.0	8.8 ± 0.1	8.9 ± 0.1	8.9 ± 0.1	8.9 ± 0.2	ns	ns	ns
Initial Weight (g)	4.9 ± 0.2	5.1 ± 0.1	5.1 ± 0.2	5.0 ± 0.2	5.1 ± 0.2	4.8 ± 0.4	ns	ns	ns
Final Weight (g)	13.0 ± 0.6	13.1 ± 0.0	12.2 ± 0.5	12.3 ± 0.3	12.2 ± 0.4	12.5 ± 0.9	ns	ns	ns
K	1.8 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.0	1.7 ± 0.1	ns	ns	ns
SGR[1] (% day^{-1})	1.2 ± 0.1	1.2 ± 0.0	1.2 ± 0.3	1.2 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	ns	ns	ns
FCR	1.2 ± 0.0	1.3 ± 0.2	1.0 ± 0.1	1.4 ± 0.2	1.5 ± 0.1	1.4 ± 0.2	**	ns	ns
DFI[1] (% day^{-1})	1.5 ± 0.1	1.5 ± 0.2	1.2 ± 0.2	1.6 ± 0.1	1.7 ± 0.1	1.6 ± 0.1	*	ns	ns
Final Mortality (%)	2.1 ± 2.6	2.2 ± 1.9	5.0 ± 4.5	4.8 ± 4.2	2.9 ± 1.4	3.9 ± 1.5	ns	ns	ns

[1]SGR and DFI data analysed using the non-parametric tests Kruskal-Wallis.
CP: crude protein; ns: not significant.

Table 4. Macronutrient composition of the whole body of ballan wrasse juveniles at the beginning of the experiment and after 70 days fed the control (D1) and experimental (D2 to D6) diets and apparent digestibility coefficients. Macronutrient data are expressed as mean ± SD (n = 3) and were analysed by two-way ANOVA (2 protein contents × 3 protein sources; * $P < 0.05$; ** $P < 0.01$). Upper case superscripts denote significant differences between the two protein contents (51 and 59), while lowercase superscripts denote significant differences between protein source (Marine, Mixed and Plant) within the same protein content treatment. Digestibility data are expressed as one value per diet as the faeces for each triplicated treatment were pooled. Wild fish and Symbio data ([1], [2]) were provided as a comparator but were not included in the statistical analysis. Carbohydrates = 100 – Ash - Crude protein - Crude lipid; ADC: apparent digestibility coefficient; CP: crude protein; DW: dry weight; ns: not significant.

Diet	Wild fish[1]	Symbio[2]	Initial sampling	D1	D2	D3	D4	D5	D6	Significance		
Protein content (% CP)		51	51	51			59			Protein content	Protein source	Interaction
Protein source		Marine	Marine	Marine	Mixed	Plant	Marine	Mixed	Plant			
Whole body composition (% DW)												
Moisture	73.7 ± 2.4	75.0 ± 0.2	78.4 ± 0.5	77.3 ± 0.1^{Aa}	77.3 ± 0.3^{Aa}	78.2 ± 0.5^{Ab}	78.0 ± 0.4^{Ba}	77.8 ± 0.3^{Ba}	78.0 ± 0.4^{Ba}	*	**	*
Ash	-	15.9 ± 0.6	15.5 ± 1.5	14.0 ± 0.2	14.8 ± 0.4	16.3 ± 1.1	13.9 ± 0.5	14.4 ± 0.4	15.2 ± 0.7	*	**	ns
Crude protein	71.1 ± 3.9	66.5 ± 1.8	71.9 ± 1.7	72.0 ± 1.1	70.7 ± 0.9	72.6 ± 1.8	73.3 ± 0.7	72.1 ± 1.3	73.0 ± 1.2	*	*	ns
Crude lipid	12.8 ± 5.7	13.1 ± 2.3	5.9 ± 0.7	12.5 ± 0.7^{b}	12.1 ± 1.3^{b}	9.1 ± 1.8^{a}	10.7 ± 0.8^{b}	10.9 ± 0.9^{b}	9.8 ± 0.9^{ab}	ns	**	*
Carbohydrate[3]	-	4.5 ± 2.1	6.7 ± 0.7	1.5 ± 0.6	2.4 ± 0.8	2.0 ± 0.8	2.1 ± 0.6	2.5 ± 1.3	2.0 ± 0.9	ns	ns	ns
Digestibility (%)[4]												
$ADC_{Proteins}$	-	47.3 ± 8.2		86.6	86.5	88.8	86.8	89.7	83.4	-	-	-
ADC_{Lipids}	-	42.9 ± 1.0		79.2	80.3	74.9	79.7	81.1	74.4	-	-	-
ADC_{Energy}	-	52.5 ± 13.3		81.2	78.2	79.2	82.7	84.3	76.4	-	-	-

[1] Hamre, 2013.
[2]Cavrois-Rogacki *et al*., 2019: ballan wrasse juveniles farmed at 16 °C and fed BioMar Symbio
[3]Calculated by subtraction
[4]Faeces for each triplicate treatment were pooled.

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Table 5. Histological assessment for inflammation level of the proximal intestine section of ballan wrasse juveniles reared for 70 days and fed the control (D1) and experimental (D2 to D6) diets. Data are expressed as mean ± SD (n = 3, 5 fish per replicate). Data were analysed by two-way ANOVA (2 protein contents × 3 protein sources; * P < .05; ** P < .01). Different superscript letters indicate significant differences between experimental groups. CP: crude protein; ns: not significant.

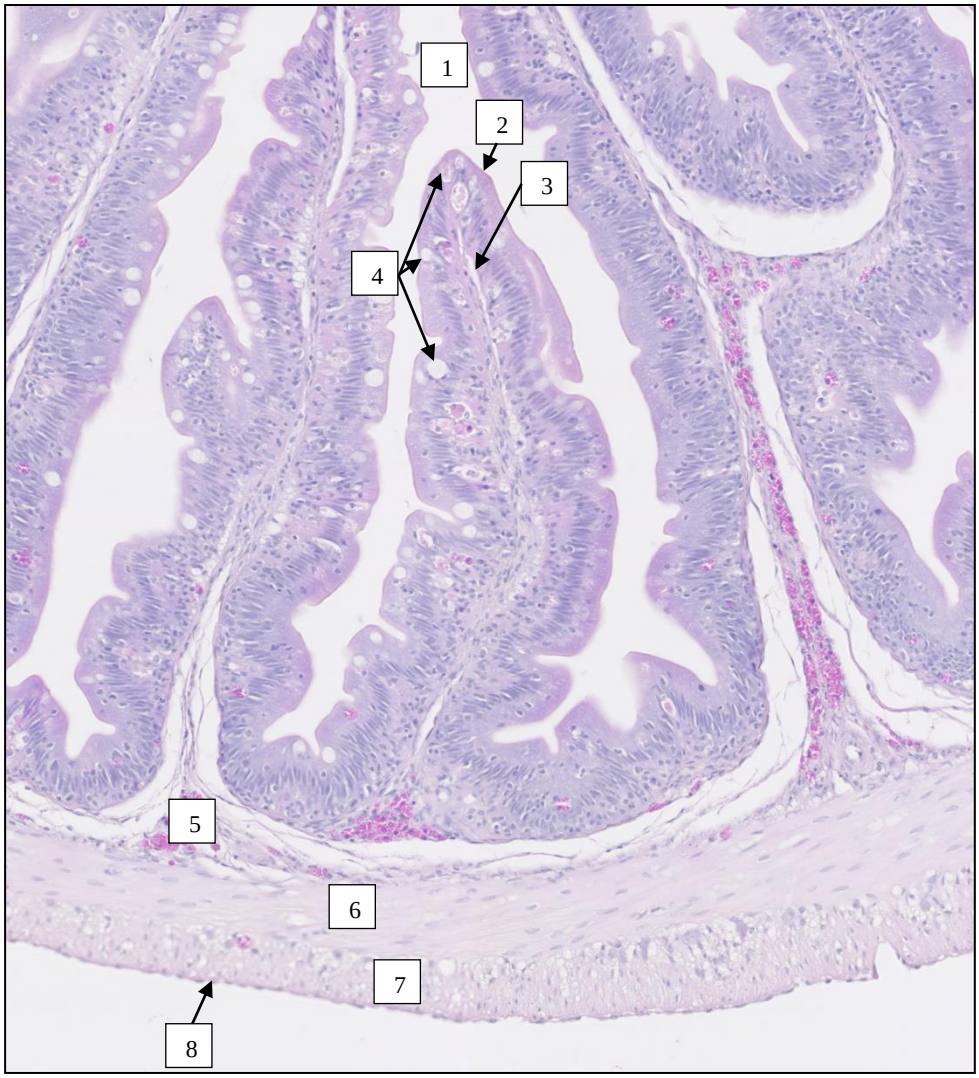
Diet	D1	D2	D3	D4	D5	D6	Significance		
Protein content (% CP)	51			59			Protein content	Protein source	Interaction
Protein source	Marine	Mixed	Plant	Marine	Mixed	Plant			
Lamina propria	1.2 ± 0.4	2.4 ± 0.9	2.2 ± 0.8	2.8 ± 0.8	1.6 ± 0.5	2.8 ± 0.4	ns	ns	**
Mucus cells	1.0 ± 0.0^{a}	1.4 ± 0.5^{a}	1.6 ± 0.5^{a}	1.8 ± 0.4^{ab}	1.2 ± 0.4^{a}	2.4 ± 0.5^{b}	*	*	*
Connective tissue	1.2 ± 0.4	1.6 ± 0.5	1.2 ± 0.4	2.2 ± 0.8	1.2 ± 0.4	1.4 ± 0.5	ns	ns	*
Mucosal folds	1.0 ± 0.0	2.2 ± 0.4	1.6 ± 0.9	2.0 ± 0.7	1.0 ± 0.0	1.4 ± 0.9	ns	ns	*
Overall score	1.1 ± 0.3	1.9 ± 0.7	1.7 ± 0.7	2.2 ± 0.8	1.3 ± 0.4	2.0 ± 0.9	*	ns	ns

Figures

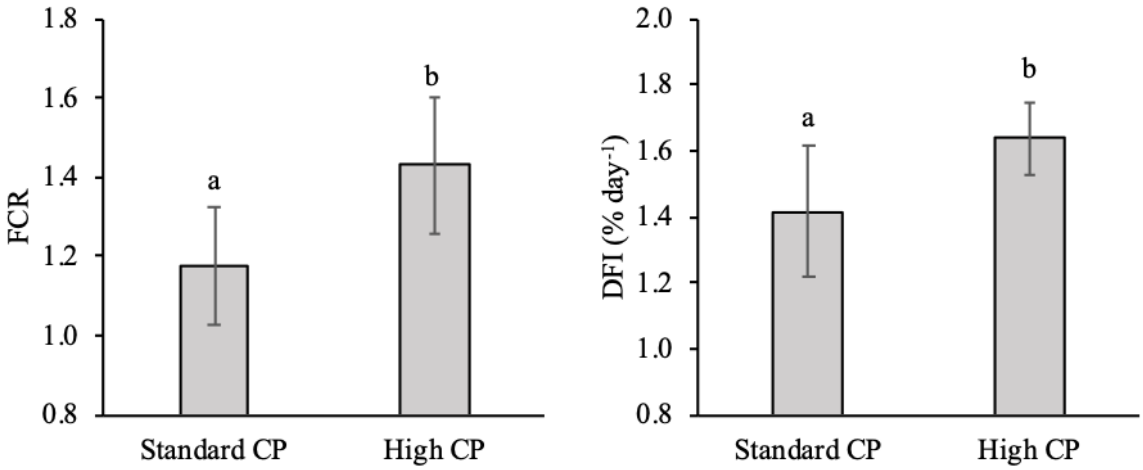
Figure 1. Transverse section of the first section of juvenile ballan wrasse intestine (x10) with structural organisation; (1) lumen, (2) columnar epithelium; (3) lamina propria; (4) mucous cells; (5) *stratum granulosum*; (6) circular muscle; (7) longitudinal muscle; (8) serosa; based on Urán *et al*. (2008).

Figure 2. Feed conversion ratio (FCR) and daily feed intake (DFI) of ballan wrasse juveniles reared for 70 days and fed the control (D1) and experimental (D2 to D6) diets. Data are expressed as mean ± SD (n = 9). Letters indicate statistical differences between treatments.

Fig. 1



712 **Fig. 2**


FCR
1.8
1.6
1.4
1.2
1.0
0.8
a
b
Standard CP
High CP
DFI (% day-1)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
a
b
Standard CP
High CP