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Adult European Seabass (*Dicentrarchus labrax*) Perform Well on Alternative Circular-Economy-Driven Feed Formulations

Jessica Petereit [1,*], Christina Hoerterer [1], Adrian A. Bischoff-Lang [2], Luís E. C. Conceição [3], Gabriella Pereira [3], Johan Johansen [4], Roberto Pastres [5] and Bela H. Buck [1,6]

1 Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany; christina.hoerterer@awi.de (C.H.); bela.h.buck@awi.de (B.H.B.)
2 Faculty of Agricultural and Environmental Sciences, Aquaculture and Sea-Ranching, University of Rostock, Justus-von-Liebig-Weg 6, 18059 Rostock, Germany; adrian.bischoff-lang@uni-rostock.de
3 SPAROS Lda, Área Empresarial de Marim, Lote C, 8700-221 Olhão, Portugal; luisconceicao@sparos.pt (L.E.C.C.); gabriellapereira@sparos.pt (G.P.)
4 Norwegian Institute of Bioeconomy Research, 1431 Oslo, Norway; johan.johansen@nibio.no
5 Dipartimento di Scienze Ambientali, Ca' Foscari University of Venice, Informatica e Statistica Via Torino 155, 30172 Mestre, Italy; pastres@unive.it
6 Applied Marine Biology and Aquaculture, University of Applied Sciences, An der Karlstadt 8, 27568 Bremerhaven, Germany
* Correspondence: jessica.petereit@awi.de; Tel.: +49-471-4831-2492

Abstract: There is an increasing need in the aquaculture industry for more sustainable and functional feed concepts for marine finfish. This study provides results for the effect of alternative feed formulations on health status, welfare parameters, sensory analysis, and growth performance in European seabass (*Dicentrarchus labrax*) over an 83-day feeding trial. Fish were fed twice a day with five experimental diets. A control diet (control) and four different alternative feed concepts rich in processed animal proteins (PAP), other alternative ingredients (NOPAP), and a positive (NOPAP^{+}) and negative (PAP^{-}) formulation were tested. All alternative formulations contained hydrolysates from aquaculture by-products and macroalgae. The results indicate that the alternative feed concepts are more sustainable alternatives compared with the commercial diet. Equally interesting, the alternative formulations did not affect the sensory analysis of the fillet quality or the animal welfare. These are increasingly important factors in aquaculture products and, accordingly, also in the formulation of new feeds. Feed concepts that are not only more sustainable in their production, have shorter transportation distances, recycle the resources (usage of by-products), and have no adverse effect on growth or welfare parameters are highly needed. Therefore, the experimental diets tested in this study are a win-win concept for future seabass aquaculture production.

Keywords: insect meal; by-products; sustainable feed; fish welfare

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1. Introduction

According to the Food and Agriculture Organization of the United Nations (FAO), more than 10% of the human population suffered from hunger in 2018, with increasing numbers over the last decade [1]. Therefore, the demand for protein sources to meet world hunger and nutritional requirements is rising. Aquaculture, as the fastest-growing, food sector holds promise for food security and offering new job opportunities [2,3]. Furthermore, it already accounts for 52% of global seafood consumption and can provide a more sustainable alternative than commercial fisheries [2].

To optimize aquaculture production while improving sustainability and ensuring the nutritional status of the fish for human consumption, it is crucial to work towards high quality, eco-friendly, and affordable feed concepts [4]. Sustainability in this paper refers to a more circular economy process that focuses on ecosystem conservation while

ensuring excellent nutritional and welfare parameters for the fish [5]. The circular economy approach is the most promising and widely used model in aquaculture. It involves the reduction and reuse of resources in production while aiming for optimization and eco-efficiency [6]. The main sustainability issue in aquaculture is the fish in/fish out ratio, which needs to be addressed to ensure a more sustainable production while minimizing resource consumption and maximizing yield [5].

Feed represents the main operational cost in marine fish farming. At present, the main protein sources are fishmeal (FM), soybean protein concentrate (SPC), wheat/corn gluten meals (WGM/CGM), and soybean meal (SBM) [7–9]. The FM, SPC, and SBM need to be augmented with alternative sources due to their high price and questionable sustainability to ensure the growth of aquaculture production [9,10]. FM is regarded as unsustainable due to finite marine resources, long transportation distances, as well as habitat degradation, and needs to be replaced to improve the sustainability of fish farming [10]. SBM is mostly produced in the American continent, in countries that often grow soy in unsustainable monocultures, which not only challenges the availability of land for food production, but also produces high transportation costs to get the meal to Europe, increasing the carbon footprint for the feed [11].

The demand for high-quality proteins in aquaculture feeds is high, and sustainable alternatives, such as plant- and terrestrial-animal-derived proteins are needed. In addition, protein hydrolysates produced from aquaculture by-products can help ensure the food safety and nutritional balance of fish while eliminating the independence of import and long transportation ways [8]. This is especially important in current situations that increase the demand for independency in the context of global pandemics (e.g., COVID-19 or other diseases), political escalations (e.g., wars or other forms of conflicts), or natural phenomena and disasters (El Nino, tsunamis, etc.) that lead to transportation bottlenecks [12]. The definition of by-products includes all raw materials (edible or inedible) that are left over from the production of the main product (here: seafood in general) and can be directly reused without further processing [5,6]. These include waste, skins, heads, blood, and bones. In fish production, an estimated 45% is used as a primary product and 55% as a by-product, highlighting the need to reuse these products to reduce environmental impact (carbon footprint with longer transportation distances) and improve production efficiency [6].

Other alternative protein sources are insect meals, plant-derived proteins, terrestrial animal by-products, fermented biomasses, and/or microalgae [10]. Taurine is deficient in plant-based protein sources, which is known to be essential for growth in carnivorous fish, such as European seabass or turbot [13,14]. It is well known that plant-derived proteins lack the nutritional benefits for feed performance in carnivorous fish and can only account for a smaller proportion in fish feed [15,16]. Simple-stomach animals, such as carnivorous fish, cannot digest plant ingredients, such as fiber. The cellulose in the feed leads to reduced energy, protein, mineral, and nutrient availability for the fish, as the complex cell walls cannot be digested [17]. Currently, we can see a steady increase in the formulated fish feed based on a mix between FM, aquaculture by-products, plant, and animal-derived proteins that lead to better digestibility of the nutrients for the fish [6,13].

Another rising issue is the mineral deficiency in fish caused by unbalanced diets that limit the mineral availability [18]. This can lead to a poor nutritional quality of the final products, so it is essential to evaluate a fish species' mineral and trace elements when looking at alternative feed ingredients [19]. It is known that carnivorous fish, such as European seabass, are hampered in the extraction of macronutrients from plants/algae [20]. This reduced availability of nutrients can cause harm in the intestinal tracts and could lead to inflammation and poor health, which is why the apparent availability needs to be considered when looking at alternative feed diets [20]. Phosphorus, calcium, iron, zinc, and iodine are the most nutritionally essential elements in marine fish, with total mineral content ranging from 0.6% to 1.5% on fresh weight [18,21]. The skeletal structure, the preservation of the colloidal system, and the regulation of acid–base equilibrium are

all critical activities of vital minerals, which are absorbed from the food and deposited in skeletal tissue and organs [21,22]. In general, fish from aquaculture have fewer toxic elements in the final products than wild fish, because wild fish bioaccumulate hazardous substances from the sea. This is another reason why aquaculture fish from RAS are a more promising solution for the safe consumption of seafood [6,22]. Welfare assessment in the aquaculture sector is becoming increasingly important for both consumers and governments, and evaluating new feed concepts in that aspect is mandatory for sustainable development [23]. For welfare assessment, it is essential to have a multilevel approach, as individual parameters are not giving an accurate evaluation of the complete state of the fish [24]. The welfare of fish is a highly complex system. The literature suggests using stress responses to assess the animals' physiological status [24–27]. Therefore, this study investigates blood parameters and immunological responses (lysozyme), known as key stress parameters [24,25,27]. European seabass is a marine fish species that is highly important in Europe for cultural as well as economic value [28,29]. As a carnivorous aquaculture species, it relies on high protein feed ingredients, usually FM, in the feed to ensure good health and growth performances [30]. It is known from the literature that FM in sea bass can be partially replaced by insect meal and plant and animal proteins without affecting the metabolism and/or growth [17,28,31,32].

This study focused not on a single ingredient analysis, but rather feed formulation concepts based on combinations of more sustainable ingredients to test for growth, nutritional, welfare, and sensory parameters. Four alternative diet compositions were tested against a commercially mimicked feed recipe to offer more sustainable feed formulations for the seabass farming industry. Feed intake, growth, feed utilization, welfare parameters, tissue composition, sensory, and mineral analysis were assessed over an 83-day feeding period.

2. Materials and Methods

2.1. Experimental Setup

A total of 375 European seabass (*Dicentrarchus labrax)* kept in the recirculation aquaculture system (RAS) of the Alfred Wegener Institute Helmholtz Center for Polar and Marine Research (AWI) (Bremerhaven, Germany) were used for the feeding experiment. The fish were acclimatized within the RAS for two weeks before starting the 83-day feeding trial. Fish were individually tagged with PIT tags and, afterwards, randomly distributed into 15 tanks (25 fish/tank) for acclimatization (Tag reader: Agrident; APR600 ISO 11784/11785 RFID Handheld Reader). The mean weight of the individuals was 320.8 ± 72.4 g. and the mean length was measured at 30.5 ± 2.1 cm, respectively.

The experimental RAS consisted of 36 individual holding tanks, with a bottom area of one m^2 and a volume of 700 L each. For all 36 tanks, the water was treated the same, using typical RAS cleaning devices, such as a drum filter, bio filter, a protein skimmer (with ozone), and a trickling filter. For this experiment, 15 out of the 36 tanks were used to ensure a density comparable to aquaculture facilities. The rest of the tanks were used as holding tanks for the spare fish.

The condition (temperature, pH, salinity, and oxygen) of the process water was monitored daily with an SC 1000 Multiparameter Universal Controller (Hach Lange GmbH, Düsseldorf, Germany), and the nutrient concentration (nitrite, nitrate, and ammonium) was measured with the QuAAtro39 AutoAnalyzer (SEAL Analytical, Norderstedt, Germany) twice a week (Table 1).

Table 1. Mean values ± standard deviation of the water parameters during the experimental trial (83 days) (temperature, pH, salinity, and oxygen: n = 83; ammonium, nitrite, and nitrate: n = 42).

Temperature (°C)	pH	Salinity	Oxygen (%)	Ammonium (mg/L)	Nitrite (mg/L)	Nitrate (mg/L)
20.0 ± 0.9	7.62 ± 0.04	36.3 ± 0.1	95.2 ± 6.7	0.096 ± 0.055	0.198 ± 0.11	127.9 ± 38

The fish were fed twice a day at 9:00 a.m. and 2:00 p.m. For the morning feeding, 50 g of pellets were provided per tank and, in the afternoon, the fish were fed *ad libitum* to ensure that the animals were fully sated. Thirty minutes after the afternoon feeding, the remaining pellets were netted and removed from the tanks, and the pellets were counted to calculate the exact amount of ingested feed.

2.2. Experimental Diets

In order to meet the nutritional requirements of European seabass and ensure good performance of the fish, the different feed compositions were prepared according to the current recommendations for this species [17,29,30]. All experimental diets, including the control, were manufactured by SPAROS LDA (Olhão, Portugal) using the same size and extrusion parameters, to minimize technological differences among the feed trials.

Based on the formulation of commercial seabass feed, four different alternative, isonitrogenous feed concepts were produced, in addition to a control diet (Table 2). All diets were extruded floating pellets with a size of 6 mm. In addition, a typical commercial diet for seabass was mimicked for the control diet, based on analysis of commercial feed labels from different commercial suppliers and further contacts with feed formulators in the industry. The no processed animal protein (NOPAP) and NOPAP^{+} diets included insect meal, fermentation biomass products, and vegetable protein concentrates; the processed animal protein (PAP) diet included poultry meal, feather meal hydrolysate, porcine blood meal, insect meal, and fermentation biomass products; and the PAP^{-} included poultry meal, feather meal hydrolysate, porcine blood meal, and fishmeal. All alternative formulations contained hydrolysates from aquaculture by-products and macroalgae. The NOPAP^{+} diet was formulated to maximize the performance of the fish and, therefore, supplemented with krill meal according to Torrecillas et al. (2021) [32]. All four alternative diets had significantly reduced levels of fish oil and rapeseed oil compared to the control; salmon oil and algae oil were used in this replacement. The NOPAP^{+} diet was used as a positive control for fish growth performance, due to a higher proportion of high-quality FM, and the PAP^{-} diet was used as a negative control, due to higher proportions of plant-derived proteins, to identify the thresholds of growth performance in *D. labrax*. Once the experimental diets were produced, they were directly delivered from Portugal to the experimental RAS facility at the AWI. Before and during the trials, the feed was stored at 4 °C to ensure continuous quality of the diets throughout the feeding experiment. The feed arrived, and the acclimatization period of the fish in the new tanks began; the feed was stored for two weeks before fish feeding started.

Table 2. Diet formulation of the experimental diets (% dry weight).

Ingredients, %	Control	NO PAP	PAP	NO PAP^{+}	PAP^{-}
Fishmeal Super Prime	10.00			15.00	
Fishmeal 60 (by-products)	5.00				
Krill meal				5.00	
Fish protein hydrolysate	3.00				
FPH-trout-head		0.50	0.50	0.50	0.50
FPH-trout-tf		0.50	0.50	0.50	0.50
FPH-turbot-head		0.25	0.25	0.25	0.25
FPH-turbot-tf		0.75	0.75	0.75	0.75
FPH-salmon-head		0.50	0.50	0.50	0.50
FPH-salmon-tf		0.50	0.50	0.50	0.50
Feather meal hydrolysate			5.00		10.00
Porcine blood meal			2.25		5.00
Poultry meal	10.00		14.00		20.00
Insect meal (*Hermetia illucens*)		15.00	10.00	10.00	
Fermentation biomass (*Corynebacterium glutamicum*)		5.00	5.00	2.50	

Table 2. *Cont.*

Ingredients, %	Control	NO PAP	PAP	NO PAP^{+}	PAP^{-}
Fermentation biomass (*Methylococcus capsulatus*)		15.00	10.00	10.00	
Soy protein concentrate	4.40				
Pea protein concentrate		2.50		3.50	
Wheat gluten	6.00	1.50		1.50	
Corn gluten meal	6.00	1.50		1.50	
Soybean meal 48	15.00				
Sunflower meal 40		9.10		6.00	13.60
Wheat meal	11.40	5.70	5.70	5.70	5.70
Whole peas	4.00	11.13	16.36	9.14	14.44
Pea starch (raw)	4.00	4.00	4.00	4.00	4.00
Vit. and Min. Premix—WITH I and Se	1.00				
Vit. and Min. Premix—NO I and Se		1.00	1.00	1.00	1.00
GAIN Macroalgae SHP		2.50	2.50	2.50	2.50
GAIN Macroalgae SHP Se-rich		0.10	0.10	0.10	0.10
GAIN Microalgae WUR Se-rich		0.20	0.20	0.20	0.20
Vitamin E50	0.03	0.03	0.03	0.03	0.03
Betaine HCl	0.10	0.10	0.10	0.10	0.10
Antioxidant	0.25	0.25	0.25	0.25	0.25
Sodium propionate	0.10	0.10	0.10	0.10	0.10
Monoammonium phosphate	1.30	2.65	1.85	1.45	1.60
L-Histidine		0.10			
L-Tryptophan	0.10	0.10	0.10		0.25
DL-Methionine	0.20	0.40	0.30	0.10	0.30
L-Taurine		0.17	0.09	0.01	0.06
Yttrium oxide	0.02	0.02	0.02	0.02	0.02
Lecithin		0.25	0.25		0.25
Fish oil	5.40	2.70	2.70	2.70	2.70
Salmon oil		9.00	9.00	13.60	9.00
Algae oil		1.00	1.00	1.00	1.00
Rapeseed oil	12.70	5.90	5.10		4.80
total	100.00	100.00	100.00	100.00	100.00

FPH: fish protein hydrolysates; I: iodine; Se: selenium; SHP: Salten Havbrukspark AS, partner that produced macroalgae; VPC: vegetable protein concentrate; YM: yeast meal; WU: Wageningen University, partner that produced microalgae.

2.3. Measurements and Sampling

At the beginning of the experiment and every four weeks, the fish were anesthetized in a bucket with 500 mg/L^{-1} tricaine methanesulphonate (MS-222; Sigma Aldrich, Taufkirchen, Germany) for three minutes. The fish were individually weighed to 0.2 g precision, and measured in length to 0.5 cm precision and identified by their tags to measure the growth performance. At the beginning of the experiment (after the acclimatization period), 15 fish were anesthetized and sacrificed to obtain baseline data for the quality of their fillet, blood, and organs. After 83 days, at the end of the entire trial, five fish per tank were sampled individually for the sampling of their tissues, organs, and blood. Additionally, five fish per tank were pooled at the end of the trial to analyze the mineral data, and, furthermore, again, five fish per tank were pooled to analyze the proximate composition. After the animals have been anesthetized, blood was taken with a heparinized syringe, and half of the blood sample was transferred to an EDTA tube with a glycolysis inhibitor (potassium). The tubes were centrifuged at 2000× *g* at 7 °C for 10 min. The plasma was pipetted into an Eppendorf tube and stored at −20 °C for further analysis. Afterwards, the fish were decapitated and tissues (liver, head kidney, and fillet without skin) were sampled rapidly and put on ice. The liver was weighted with 0.1 mg precision to determine the hepatosomatic index (HSI). All tissues were shock frozen in liquid nitrogen and stored at −80 °C until further analysis. For digestibility analysis, the feces were sampled by a collection device per one tank (meaning three tanks per diet and 15 tanks in total). The collection device was installed under each tank and was emptied before the feeding in the morning and collected after a 4 h digestion period. The feces were centrifuged at 4° C and 3000× *g* for 5 min, and the supernatant was removed and frozen at −80 °C until further analysis. For the fillet analysis,

five fish per tank were stunned with a head blow and then killed with a gill cut. All fillets were removed and weighted for the total yield and frozen at −20 ° C for further analysis.

2.4. Homogenization of Diets, Whole Body, and Feces

The diets were homogenized in a knife grinder (5000 rpm, 30 s, Grindomix GM 200, Retsch, Germany) two times to achieve homogenized materials and deep-frozen for further analysis. The collected feces were pooled per tank (n = 15) and freeze-dried for 24 h. Afterwards, the freeze-dried samples were homogenized in a knife grinder (5000 rpm, 30 s, Grindomix GM 200, Retsch, Germany) and stored at −20 °C for further analysis.

Whole fish samples were pooled from three individuals. The fish were minced frozen in a commercial meat grinder, refrozen at −20 °C, and then freeze-dried for 48 h.

2.5. Chemical Analysis

2.5.1. Moisture, Ash, and Energy Analysis

The moisture content and ash of the experimental diets, feces, and whole-body fish was determined after AOAC (1980). The moisture content of the feeds was determined by drying the samples at 105 °C for 24 h. The moisture content of the feces and whole body was determined by freeze-drying for 24 h for the feces and 48 h for the whole-body samples. Total ash content was determined by combustion of the samples in a muffle oven at 550 °C for 6 h. Gross energy was measured in an adiabatic bomb calorimeter (Model 6100, Parr Instrument, Frankfurt am Main, Germany).

2.5.2. Crude Fat and Crude Protein Analysis

The crude fat and crude protein analysis of the feed, as well as the whole-body samples, were conducted in an external lab (Labor IBEN GmbH, Bremerhaven, Germany). The crude fat was analyzed after Weibull/Stoldt (ASU L 06.00-6 2014-08* (Modification: Extraction with Soxtherm) and the crude protein with N × 6.25 (ASU L 06.00-7 2018-06*). For all other samples (feed, carcass, and fillet), the measured total nitrogen was converted to equivalent crude protein (%) using a conversion factor of 6.25. Crude lipid was determined after Weibull-Stoldt.

2.5.3. Mineral Analysis

The mineral analyses of diets, feces, and whole-body composition were conducted in duplicates. For the analysis of the mineral content, 0.2 g of freeze-dried and homogenized samples of the experimental diets, feces, and whole body were digested in 3 mL nitric acid HNO_3 (65%, trace grade) in a microwave oven (CEM MARS5, Kamp-Lintfort, Germany) according to DIN EN 13805 (2014). After digestion, the samples were diluted with milli-q water to 50 mL. Calcium, potassium, magnesium, phosphorus, arsenic, copper, iron, manganese, yttrium, and zinc concentrations were analyzed in an ICP-OES (iCAP7400, Fisher Scientific, Schwerte, Germany). Fish muscle (ERM—BB422, EU) was used as reference.

2.6. Blood Parameters

All blood parameters, except for the lysozyme activity, were analyzed in an external lab in Bremerhaven, Germany (Labor Dr. Schumacher MVZ GmbH, Bremerhaven, Germany). The following parameters were measured: potassium, sodium, calcium, lactate dehydrogenase (LDH), total proteins, and glucose. LDH, glucose, and total protein were analyzed with a photometer at 700 nm. The total protein content was analyzed after the biuret method and glucose after the hexokinase method; for LDH, lactate was transformed into pyruvate (IFCC method). Potassium, natrium, and calcium were analyzed with an ion-selective electrode method, in which a selective membrane measures the ions of each parameter.

Lysozyme Activity

The lysozyme activity was performed to the protocol of Milla [33]. The phosphate buffer consisted of 0.05 mol/L NaH_2PO_4 + 0.05 mol/L Na_2HPO_4 and was modified with 85% H_3PO_4 to a pH of 6.2. A total of 30 mg of *Micrococcus luteus* (0.6 mg/mL, SIGMA

M3770) was mixed with 50 mL buffer on a daily basis, while 20 mg lysozyme from egg whites (Lot SLCC4285, 40382 Units/mg, Sigma L6876) was mixed with 20 mL of buffer weekly. The lysozyme–buffer solution was diluted to obtain 1000 U/mL. Therefore, 248 µL lysozyme solution was buffered in 9.752 mL buffer.

A standard curve was created that ranged from 100 U/mL to 900 U/mL, as well as two blank samples (one with the bacterium and one with the buffer). For the samples, 10 µL and 5 µL of plasma with 10 µL and 15 µL of buffer were pipetted into the wells to obtain a volume of 20 µL per well. Immediately before starting the measurement, *M. luteus* was added at 130 µL to the standard curve and all sample preparations. For the measurement, 96-well plates (Brandplate 781660) with a clear flat bottom were used and measured in a Berthold Tristar LB941.

The measurement was performed at 450 nm every minute over a period of 10 min. The plate was shaken for 5 s before the first measurement. For each measurement, the standard curve, samples, and blanks were measured in triplicate. Between measurements, the solutions were stored as much as possible in the refrigerator at 4 °C and the samples in the freezer at −20 °C.

2.7. Fillet Analysis

Sensory Analysis

The sensory analysis of the fillet was conducted in an external lab (Labor IBEN GmbH, Bremerhaven, Germany). The fillets were tested for their consistency before and after cooking, smell, taste, color, juice and grease separation, and protein precipitation with an ASU l 0.90-6 2015-06 standard Norm method. The sensory analysis was carried out in the sensory room of the Labor IBEN by at least two test persons. The testers describe the test samples individually or jointly using descriptive expressions of their choice or based on predefined lists (for fresh fish, for example, according to the Karlsruher Scheme).

The samples were cooked, and the temperature of the test samples was the same for each test person at the time of presentation.

The testing room was clean and odor-free at all times. Lighting was uniform, glare-free, and as close to the daylight spectrum as possible. The appearance, odor, taste, and consistency of the food were assessed.

2.8. Calculation and Formulas

The growth parameters were based on body weight (*BW*) and body length (*BL*) and was calculated as follows:

$$Weight\ gain\ (WG)\ (\mathrm{g}) = BW\ final\ (\mathrm{g}) - BW\ initial\ (\mathrm{g}) \quad (1)$$

$$Relative\ growth\ rate\ (RGR,\ \%) = 100\ (e^{\frac{\ln(BW\ final\ (\mathrm{g}) - \ln(BW\ initial\ (\mathrm{g})))}{feeding\ days}} - 1) \quad (2)$$

$$Condition\ factor\ (CF\ \%) = 100 \times \frac{BW\ final\ (\mathrm{g})}{BL\ final^3(\mathrm{cm})} \quad (3)$$

$$Hepatosomatic\ Index\ (HSI\ \%) = 100 \times \frac{liver\ weight\ (\mathrm{g})}{BW\ final\ (\mathrm{g})} \quad (4)$$

$$Viscerosomatic\ Index\ (VSI\ \%) = 100 \times \frac{viscera\ weight\ (\mathrm{g})}{body\ weight\ (\mathrm{g})} \quad (5)$$

The feed performance parameters, daily feed intake (*DFI*), and feed conversion ratio (*FCR*) were based on the feed intake (*FI*) in g of the offered amount of feed and the uneaten feed. Total FI and WG for FCR were corrected for the lost biomass through mortalities and sampling.

$$Total\ feed\ intake\ (F)\ total\ (\mathrm{g}) = (Feed\ offered) - (Feed\ uneaten) \quad (6)$$

$$Daily\ feed\ intake\ (DFI,\ \%\ BW == 100 \times \frac{FI\ total\ (g)}{(Feeding\ days \times \frac{(BW\ final + BW\ initial)}{2})} \quad (7)$$

$$Feed\ conversion\ ratio\ (FCR) = \frac{FI\ total\ (g)}{WG\ (g)} \quad (8)$$

$$Protein\ efficiency\ ratio\ (PER) = \frac{weight\ gain\ (g)}{crude\ protein\ intake\ (g)} \quad (9)$$

The apparent digestibility (*ADC*) of the dietary nutrients and the apparent availability (*AA*) of minerals were based on the amount of the inert yttrium marker in the diet and feces and the respective nutrient or element in feces and diets.

$$ADC\ dry\ matter\ (\%) = 100 - (100 \times \frac{yttrium_{diet}}{yttrium_{faeces}}) \quad (10)$$

$$ADC\ nutrient\ (\%) = 100 - \left(100 \times \left(\frac{yttrium_{diet}}{yttrium_{faeces}} \times \frac{nutrient_{faeces}}{nutrient_{diet}}\right)\right) \quad (11)$$

$$AA\ (\%) = 100 - \left(100 \times \left(\frac{yttrium_{diet}}{yttrium_{faeces}} \times \frac{element_{faeces}}{element_{diet}}\right)\right) \quad (12)$$

2.9. Statistical Analysis

Statistical analysis was conducted with Sigma Plot (12.5, Systat Software, Erkrath, Germany). One-way analysis of variance (ANOVA) was used to determine significant differences between the treatments. Whenever there were statistically significant differences, an all pairwise multiple comparison procedure was performed using the Holm–Sidak method overall significance level $p < 0.05$ to find the difference within the treatments. Values are given as means ± standard deviations. The sensory analysis was an ANOVA on ranks performed to see the statistical difference ($p < 0.05$). If statistical differences were detected, all pairwise multiple comparison procedure (Tukey test) was performed to detect differences over the significance level between the treatments.

3. Results

3.1. Feed

The proximate composition of feed can be seen in Table 3. The moisture was significantly higher in the PAP^{-} and NOPAP^{+} diets. The energy was significantly lower in the PAP diet and dry matter was significantly lower in the PAP and PAP^{-} diet compared to the control diet (Table 3).

Table 3. Chemical composition of the experimental diets.

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Ash (%)	7.98 ^{b}	8.10 ^{ab}	8.23 ^{a}	8.06 ^{b}	8.04 ^{b}	0.039
Moisture (%)	8.10 ^{c}	8.88 ^{d}	8.88 ^{d}	10.19 ^{b}	11.30 ^{a}	<0.001
Gross energy (MJ kg^{-1})	23.16 ^{a}	21.58 ^{b}	22.88 ^{a}	23.08 ^{a}	23.14 ^{a}	<0.001
Crude protein (%)	37.9	39.0	38.5	35.8	41.0	0.406
Crude fat (%)	22.2	22.2	21.8	21.2	21.5	0.406
Apparent digestibility coefficient						
Dry matter (%)	79.18 ± 0.93 ^{ac}	77.59 ± 1.13 ^{bce}	76.24 ± 1.88 ^{acd}	76.14 ± 2.56 ^{de}	80.52 ± 1.01 ^{ab}	0.032
Gross energy (%)	74.95 ± 1.46	75.143 ± 1.17	73.60 ± 1.46	73.43 ± 1.53	70.48 ± 6.56	0.440

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA.

The mineral composition of the feed can be seen in Table 4. All diets had an yttrium marker inside their formulation to later be able to analyze the apparent digestibility (ADC) of the different nutrients/minerals. The minerals were tested without replicates, as prior analysis determined very small variations within the mineral analysis of feed.

Table 4. Mineral composition of the experimental diets.

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}
Calcium (Ca; g/kg^{-1})	16.68	18.12	17.75	16.70	17.07
Potassium (K; g/kg^{-1})	6.72	6.43	6.51	5.41	6.44
Magnesium (Mg; g/kg^{-1})	1.82	1.71	1.77	1.57	1.67
Sodium (Na; g/kg^{-1})	4.04	3.73	3.85	2.31	4.47
Phosphorus (P; g/kg^{-1})	13.27	13.42	13.47	13.58	13.02
Ca/P ratio	1.26	1.35	1.32	1.23	1.31
Copper (Cu; mg/kg^{-1})	17.36	17.50	18.86	18.87	17.42
Iron (Fe; mg/kg^{-1})	346.04	198.41	180.04	259.99	162.49
Manganese (Mn; mg/kg^{-1})	34.73	32.38	34.39	37.78	25.51
Zinc (Zn; mg/kg^{-1})	173.97	161.39	171.89	181.72	179.55

3.2. Growth and Feed Performance

There were no mortalities during the feeding experiment. The fish fed the alternative diets showed no significant differences in total feed intake, FCR, or condition factor (Table 5).

Table 5. Performance parameters of the seabass fed five experimental diets (n = 45).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Total feed intake (g)	261.92 ± 16.11	254.55 ± 5.76	247.27 ± 10.87	242.97 ± 8.22	258.85± 4.23	0.349
FCR	1.74 ± 0.04	1.96 ±0.16	1.67 ± 0.07	1.92 ± 0.17	1.79 ± 0.14	0.213
CF	1.20 ± 0.03	1.19 ± 0.02	1.19 ±0.03	1.19 ±0.01	1.21 ±0.02	0.817

Values are expressed as means ± SD, *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

All fish had an initial weight of 320.8 ± 72.4 g. The weight gain at the end of the 83-day trial was significantly higher in control fed fish compared to the PAP and NOPAP treatments, as well as between PAP^{-} and PAP fed fish (*p* value: 0.035).

The health indicator hepatosomatic index (HSI) showed no significant difference between the treatments, but the viscerosomatic index (VSI) differs significantly between the PAP^{-} fed fish and the NOPAP^{+} and control fed fish (*p* value: 0.009) (Table 6). The relative growth rate was significantly higher in control fed fish compared to all alternative diets: PAP^{-}, NOPAP, PAP and NOPAP^{+} (*p* value: 0.009).

Table 6. Growth and health performance parameters of the seabass fed five experimental diets (n = 45).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Weight gain (g)	159.90 ± 127.9 ^{a}	148.20 ± 129.2 ^{a}	144.00 ± 118.6 ^{bc}	138.55 ± 116.5 ^{bc}	144.50 ± 125.6 ^{ac}	0.035
HSI (%)	1.52 ± 0.37	1.41 ± 0.25	1.46 ± 0.26	1.47 ± 0.35	1.63 ± 0.32	0.438
VSI (%)	9.33 ± 1.52 ^{a}	8.97 ± 1.82 ^{a}	8.54 ± 1.42 ^{a}	8.30 ± 1.18 ^{b}	9.73 ± 1.77 ^{a}	0.009
BW final (g)	466.30 ± 105.22	475.68 ± 102.95	460.18 ± 88.28	459.33 ± 104.01	474.78 ± 101.47	0.847
RGR (%/day)	0.52 ± 0.13 ^{a}	0.48 ± 0.13 ^{b}	0.46 ± 0.09 ^{b}	0.45 ± 0.11 ^{b}	0.47 ± 0.09 ^{b}	0.009

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

3.3. Whole-Body Composition

The whole-body analysis showed no significant differences between the crude fat, moisture, ash, or PER between all groups. However, the crude protein was significantly higher in the NOPAP fed diet compared to the PAP^{-} and NOPAP^{+} (p value: 0.022) fed fish (Table 7). The energy content of the NOPAP^{+} fed fish was significantly higher compared to control, PAP, or PAP^{-} fed fish (p value: 0.046).

Table 7. Whole-body composition parameters of seabass fed five experimental diets (n = 15).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	p Value
Moisture (%)	37.69 ± 2.33	37.45 ± 3.92	36.57 ± 2.83	38.61 ± 2.04	41.49 ± 7.05	0.296
Ash (%)	10.29 ± 1.10	10.42 ± 0.83	10.20 ± 0.64	10.45 ± 0.57	11.28 ± 3.71	0.845
Energy (MJ kg^{-1})	25.40 ± 1.37 ^{b}	25.11 ± 1.09 ^{b}	26.44 ± 1.92 ^{ab}	25.10 ± 1.31 ^{b}	29.90 ± 5.95 ^{a}	0.046
Crude fat (mg/kg)	16.47 ± 0.72	15.6 ± 0.7	16.37 ± 1.10	15.87 ± 2.20	15.45 ± 0.35	0.841
Crude protein (mg/kg)	18.03 ± 0.15 ^{a}	18.03 ± 0.15 ^{a}	18.57 ± 0.21 ^{b}	17.83 ± 0.40 ^{a}	17.75 ± 0.07 ^{a}	0.022
PER	1.56 ± 0.05	1.35 ± 0.11	1.56 ± 0.08	1.48 ± 0.11	1.36 ± 0.12	0.057

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), p values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

3.4. Mineral Analysis

The mineral content of the whole body showed no significant differences for the cultured seabass in any of the analyzed mineral and trace elements (Table 8).

Table 8. Analyzed mineral concentration on a wet weight basis in the whole body of seabass fed with five experimental diets for 83 days (n = 15).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	p Value
As (mg/kg)	0.0004 ± 0.00	0.0001 ± 0.00	0.0003± 0.00	0.0003 ± 0.00	0.0002 ± 0.00	0.075
Ca (g/kg)	29.67 ± 2.61	28.67 ± 3.36	30.42 ± 0.45	33.61 ± 5.95	26.45 ± 4.14	0.287
Cu (mg/kg)	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000	0.002 ± 0.001	0.003 ± 0.000	0.124
Fe (mg/kg)	29.47 ± 3.69	30.59 ± 2.37	28.82 ± 7.44	28.96 ± 4.02	23.55 ± 6.33	0.522
K (g/kg)	9.26 ± 0.65	9.23 ± 0.25	9.00 ± 0.31	9.12 ± 0.53	9.26 ± 0. 15	0.924
Mg (g/kg)	1.06 ± 0.07	1.08 ± 0.03	1.04 ± 0.04	1.08 ± 0.11	1.03 ± 0.05	0.825
Mn (mg/kg)	6.42 ± 0.85	6.01 ± 0.79	5.71 ± 0.51	6.60 ± 1.30	5.12 ± 0.93	0.349
Na (g/kg)	3.12 ± 0.29	3.05 ± 0.13	2.99 ± 0.15	3.21 ± 0.23	3.03 ± 0.01	0.646
P (g/kg)	18.85 ± 1.58	18.48 ± 1.58	18.34 ± 1.09	20.55 ± 3.18	17.25 ± 1.95	0.422
Zn (mg/kg)	37.43 ± 0.83	36.54 ± 1.80	37.75 ± 2.89	37.84 ± 2.54	34.15 ± 2.57	0.302

Values are expressed as means ± SD, p values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

The apparent availability of sodium was significantly lower in PAP^{-} fed fish than in all other fed diets (p value: <0.001). Iron availability was significantly lower in the NOPAP^{+} and PAP fed fish compared to control and PAP^{-} (p value: 0.021) fed fish. However, the PAP^{-} fed fish showed significantly higher zinc availability compared to control, NOPAP, and NOPAP^{+} (p value: 0.043) fed fish (Table 9).

Table 9. Apparent availability of minerals and trace elements on a wet weight basis in the whole body of seabass fed with five experimental diets for 83 days (n = 15).

Apparent Availability (%)	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Calcium (Ca)	21.33 ± 8.3	27.47 ± 11.92	34.22 ± 14.58	26.60 ± 12.90	14.54 ± 7.20	0.346
Potassium (K)	87.79 ±1.33	88.19 ± 0.29	88.04 ± 0.91	85.96 ± 1.83	87.82 ± 0.59	0.180
Magnesium (Mg)	−130.35 ± 0.81	−145.74 ± 29.20	−120.79 ± 22.04	−164.99 ± 32.26	−149.89 ± 2.85	0.193
Sodium (Na)	−298.73 ± 46.47 ^{a}	−295.86 ± 13.90 ^{a}	−276.14 ± 18.36 ^{a}	−545.01 ± 92.41 ^{b}	−229.73 ± 19.68 ^{a}	<0.001
Phosphorus (P)	55.59 ± 1.70	60.35 ± 8.20	60.62 ± 7.25	66.40 ± 7.70	59.47 ± 6.08	0.438
Copper (Cu)	67.26 ± 4.10	63.22 ± 5.75	67.03 ± 5.18	69.12 ± 7.85	65.17 ± 5.40	0.770
Iron (Fe)	23.82 ± 22.54 ^{a}	−2.84 ± 5.56 ^{bc}	7.75 ± 6.47 ^{ac}	25.66 ± 6.35 ^{a}	−5.07 ± 5.79 ^{bc}	0.021
Manganese (Mn)	31.59 ± 12.67	37.67 ± 19.18	43.48 ± 15.18	55.45 ± 10.1	23.29 ± 6.01	0.112
Zinc (Zn)	19.99 ± 3.24 ^{a}	31.45 ± 11.75 ^{ab}	26.40 ± 11.24 ^{a}	46.11 ± 9.78 ^{b}	21.22 ± 9.16 ^{a}	0.043

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

3.5. Welfare Parameters

The plasma analysis showed no significant differences in glucose, lysozyme, and LDH activity. The total protein content in plasma was significantly lower between the NOPAP^{+} fed fish and the control, NOPAP, and PAP- fed fish, as well as between PAP^{-} and control fed fish (*p* value: 0.001) (Table 10).

Table 10. Main blood parameters of seabass fed with experimental diets for 83 days (n = 15).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	*p* Value
Lactatdehy-drogenase (U/L)	46.44 ± 32.49	51.84 ± 32.52	43.88 ± 33.51	57.58 ± 28.53	41.76 ± 27.43	0.226
Glucose (mmol/L)	91.36 ± 26.60	95.72 ± 24.06	87.67 ± 17.69	85.96 ± 23.29	94.24 ± 29.36	0.384
Total protein (g/L)	41.68 ± 4.08 ^{a}	40.07 ± 4.13 ^{ac}	40.07 ± 3.36 ^{ac}	38.68 ± 3.59 ^{bc}	36.76 ± 3.44 ^{b}	0.001
Lysozyme (U/mL)	290.33 ± 34.46	270.67 ± 49.53	275.96 ± 44.33	276.17 ± 77.07	294.77 ± 70.16	0.169

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

The fatty acids palmitinacid, linoleicacid, linolenic acid, and DHA showed no significant differences between the fish fed different diets. However, oleic acid was significantly lower in the fish fed the PAP diet compared to the control and PAP^{-} fed fish (*p* value: 0.016) (Table 11).

Table 11. Main fatty acids of seabass fed with five experimental diets for 83 days (n = 15).

Feed/Fatty Acid (mg/g)	Control	PAP	NOPAP	PAP^{-}	*p* Value
Palmitinacid (16:0)	27.73 ± 13.67	25.29 ± 20.65	29.22 ± 24.86	27.90 ± 11.8	0.950
Oleic acid (18:1n–9)	46.86 ± 28.64 ^{a}	22.84 ± 10.17 ^{bc}	36.47 ± 28.64 ^{ac}	48.01 ± 18.36 ^{a}	0.016
Linoleicacid (18:2)	19.30 ± 10.69	16.79 ± 14.92	18.89 ± 11.48	19.80 ± 8.46	0.899
Linolenic acid(18:3)	4.97 ± 3.69	4.36 ± 4.54	5.53 ± 3.84	5.41 ± 2.84	0.830
EPA (20:5)	7.12 ± 2.55	5.53 ± 1.98	6.25 ± 2.19	5.51 ± 1.43	0.123
DHA (22:6)	8.72 ± 3.46	8.53 ± 2.08	10.07 ± 3.31	8.26 ± 1.76	0.306

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), *p* values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

3.6. Sensory Analysis

A significant difference (p value: 0.001) could be seen in fillet weight between the fish fed the NOPAP^{+} diet compared to the control and all other alternative diets at the end of the trial (Table 12). Sensory analysis showed no significant differences in any of the tested parameters: consistency frozen fat separation, protein, juice separation, or taste (one-way ANOVA on ranks; Tukey test).

Table 12. Fillet yield (%) of seabass fed with five experimental diets for 83 days (n = 15).

Feed	Control	PAP	NOPAP	PAP^{-}	NOPAP^{+}	p Value
Filet weight (%)	37.89 ± 2.18 ^{b}	39.24 ± 2.87 ^{b}	37.60 ± 2.51 ^{b}	39.03 ± 2.02 ^{b}	41.20 ± 2.15 ^{a}	0.001

Values are expressed as means ± SD, values with different letters within the same line are significantly different ($p < 0.05$), p values from one-way ANOVA. Control: commercial formulation, PAP: processed animal-protein-rich diet, NOPAP: insect-, fermentation-, and plant-based protein-rich diet. PAP^{-}: more plant-based materials (used as negative control); NOPAP^{+}: similar to NOPAP but enriched with high-quality fishmeal and krill meal (used as positive control).

4. Discussion

The present study examined whether the replacement of fishmeal (FM), fish oil (FO), and soy products in feed efficiency by alternative ingredients affect the performance of European seabass (*Dicentrarchus labrax*). Different formulation concepts were tested including ingredients, such as processed land-animal proteins, vegetable protein concentrates, insect meal, fermentation products, macroalgae, fish protein hydrolysates from aquaculture by-products, salmon oil, and algae oil. The literature shows that seabass is known to have good acceptability of plant and animal-derived protein replacements at moderate levels [17,30,33,34]. The present study attempts to test whole feed alternative formulation concepts, with the replacement of more than one ingredient at a time. In the following, the different performance aspects are considered to better interpret the general health and growth parameters, first from a physiological point of view and later from the perspective of the consumer and/or farmer. The distinction between aquaculture candidate and farmer is critical here, as the farmer has to consider the best aspects for the welfare of the fish as well, while, on the other side, being economically feasible. For the consumer, it is the sensory analysis, as well as food safety in terms of toxic elements and healthy fatty acids.

4.1. Growth and Feed Performance

The present study examined the effect on growth and feed performances, and no significant differences were found in total feed intake, FCR, CF, or final body weight for all different diets. In contrast, the relative growth rate was significantly higher in the control fed fish (0.52%/day) than in all other diets (0.45–0.48%/day). The same could be seen for the weight gain, which was significantly higher in the control and PAP fed fish compared to the other diets. The significant weight gain but insignificant final weights can be explained by the high variability in the initial weights.

Health indicators, such as viscerosomatic index (VSI) (7–13%) and hepatosomatic index (HSI) (1.5–2.3%), are congruent with the recent literature [19]. The HSI (1.4–1.6%) showed no significant differences between the different treatments, indicating good health performance for all feeding concepts. The VSI, on the other hand, shows significantly lower values for the PAP fed fish (8.3 ± 1.18) compared to all other feed concepts (8.54–9.73). This result was expected as it is already described in the literature that more plant-based diets can lower health performances in carnivorous fish [13,15–17]. Comparing the different feed concepts and growth parameters, we see that the control and PAP fed fish had the best performance, closely followed by the NOPAP^{+} and NOPAP fed fish, and PAP fed fish with the lowest performance. Overall, these results indicate that the control diet can be exchanged for one of the more sustainable alternative diets (PAP or NOPAP) with no adverse effect on growth. This is in line with other studies investigating European seabass using less radical alternative formulations [19,30,35].

4.2. Whole-Body Composition

The whole-body composition did not show significant differences in moisture and ash between the control and alternative diets and is congruent with the recent literature [19,23,33]. Crude fat (here: 15.45–16.47 mg/kg) is well within the normal range for European seabass (7–21 mg/kg) and does not significantly differ between the different feed concepts [19,28,33,36]. Crude protein is significantly higher between the NOPAP fed fish (18.57 mg/kg) and the other feed concepts (17.75–18.03 mg/kg) but still well within the range of the literature (15–22 mg/kg) [19,28].

The protein efficiency ratio (PER) range of 1.35–1.56 is well within the range described by the literature and shows a good ratio within all experimental and control fed fish [37]. Higher lipid levels in the diet are already known to improve feed efficiency and PER [37]. Fish that obtained the highest PER in this study were fed the commercial and NOPAP diet, with lipid levels of 22.2% and 21.8%. The literature shows a decrease in PER in seabass with increasing dietary protein beyond the optimum level [37–39]. This can be observed in the PER of the PAP and NOPAP^{+} fed fish, which had crude fat levels of 39% and 41%.

These findings are congruent with the literature that shows no body compositional decrease by plant- [34] and terrestrial-animal-derived protein replacements of up to 30% [19,28,40]. The conversion factor used in this study was an estimate for all diets. In an experimental design, such as the one described here, with feed concepts rather than individual ingredients, the exact conversion factor calculation is complex and not feasible for the farmers. That is why we determined the factor nutritionally, which gives a good overview of the respective concepts. Therefore, it was concluded that the applied alternative feed concepts do not negatively affect the biochemical composition of seabass.

4.3. Mineral Analysis

No significant differences in mineral concentrations were found between control and replacement feed concepts, which is congruent with the literature replacing fishmeal up to 33% [18,41,42]. Furthermore, low values of feed-derived toxic elements, such as arsenic, were found in all fish, independent of the feed concept. Arsenic is known to impair growth, especially in children, and the lower the value, the less toxicity can be assumed, leading to safer end products [22,30]. Since wild fish contain higher amounts of arsenic (1.030–1.230 mg/kg), fish fed with the experimental diets are safer for human consumption, an important positive aspect in cultured fish [36]. Interestingly, a significant decrease in arsenic in the PAP fed fish can be seen, which could be interpreted as a better option for human health than the control diet fed fish. This study indicated that iron and zinc (in the ranges of 23–30 and 34–37 mg/kg, respectively) were the main micro-mineral components, similar to that observed in other studies in cultured seabass [4]. Zinc is known to have significant healing, antioxidant, and immunostimulating properties in carnivorous fish, and a high availability, such as in the fish of this study, is wanted to ensure high health standards [43]. In general, copper, iron, and zinc are known to be nutritionally vital metals and can have positive effects on human health [18].

Plant-based feed ingredients are known to bind minerals and, thus, reduce the availability of phosphorus, iron, and zinc in fish [44]. In this study, no negative correlation between the alterative and more sustainable feed concepts (PAP and NOPAP) could be seen. However, it is known that calcium, phosphorus, and manganese are mainly accumulated in fish bones, heads, and gills, meaning a higher % of fishmeal leads to elevated levels of the listed minerals [18]. This study showed that the replacement of FM with aquaculture by-products in the presented feed concepts does not negatively affect the amount of the above minerals and is, thus, a good and more sustainable alternative than the commercial FM.

In general, it can be said that the ingredients in feed can have two opposing effects: (1) supplying minerals and (2) supplying substances that reduce the absorption of minerals [45]. Therefore, the availability of nutrients can be explained as the total of these two effects. When the latter effect is larger, the availability is somewhere below 0%, resulting in negative values like in sodium and magnesium. Our study showed that higher proportions

of bone materials, such as in the PAP^{-} diet, lead to reduced availability of the total amounts of minerals [45].

Since the mineral concentrations in the whole body are similar in the fish from all diet concepts, it can be concluded that the mineral and trace element demand was sufficiently covered and that elevated excretion rates balanced the elevated mineral concentration in the experimental diets. Therefore, fish are a good source of essential minerals, and this study showed that the alternative diets tested here do not negatively affect the mineral composition and can replace up to 17% of FM [42].

4.4. Welfare Parameters

Among others, plasma metabolites, such as glucose and total protein, are considered as essential and sensitive indicators for the physiological changes due to stress and diseases [46]. In this study, plasma glucose (41.7–57.6 mmol/L), LDH (41.76–57.58 U/L), and lysozyme levels (270–294 U/mL) were not affected by alternative diet fed fish, congruent with the literature where up to 15% of plant material was replaced without adverse effect [17,23,24,33]. In contrast to that, a decrease in total protein content can be seen between control (41.68 g/L) and PAP^{-} (38.68 g/L)/$NOPAP^{+}$ (36.76 g/L) fed fish. These values are congruent with the literature and may imply that seabass is either even more tolerant of substandard feed mixtures, especially PAP^{-}, or that the $NOPAP^{+}$ mixture performed below expectations and targets in terms of, e.g., bioavailability of nutrients [47]. The total protein content in the plasma can be seen as a health indicator, as an elevated level would indicate a poor immune system. The low values in the PAP^{-} diet were expected and can be overlapped with the low VSI, another indicator for poor health. The welfare status is a very complex system, and many factors need to be considered to understand the picture entirely. For the $NOPAP^{+}$ diet, we can see a lower value on protein content in the plasma, but none of the other measured parameters indicate poor welfare. This concludes that welfare is not necessarily negatively affected in $NOPAP^{+}$ fed fish. The lower values in the total protein in the plasma are more likely explained by the fact that too much protein content in the feed leads to less protein efficiency, as can be proven by the low PER in $NOPAP^{+}$ fed fish. The sustainable alternative diets, PAP and NOPAP, induced no significant differences in the blood parameters and can, thus, be considered to provide a similar welfare status of the fish when exchanged for the less sustainable control diet.

When looking at the welfare status of fish, it is crucial to take highly unsaturated fatty acids (HUFAs) into account, which are mainly responsible for the metabolism of fish and generate growth and immune function [28,48]. Furthermore, especially in farmed animals, higher stress is given due to handling, densities, and transportation, which make the health status of fish even more essential, and amino acid need to be considered a critical welfare category. Values for all fatty acids were within the range of European seabass and showed no significant differences for any fatty acids, except the oleic acid, compared to the control fed fish vs. alternative diet fed fish [18,49]. Palmitic acid between 12 and 29 mg/g, oleic acid between 31 and 44 mg/g, linoleic acid between 7 and 35 mg/g, linolenic acid between 1 and 5 mg/g, EPA between 1.5 and 7 mg/g, and DHA between 4 and 13 mg/g were well within the range of the literature and do not indicate poor welfare status [18,49]. The most important fatty acids for human consumption are linolenic acid, EPA, and DHA, which are well-researched and function in important metabolic processes for human health and are well-expressed in fish from this study, supporting fact for safe and eco-friendly seafood [18,49,50]. In today's society, healthy food is critical, as more and more contaminants (whether vegetables or meat) are found, and consumers are confused in their purchasing decisions. Overall, the welfare parameters are not negatively affected when the control diet is replaced by one of the more sustainable feed concepts with processed animal (PAP) or plant proteins (NOPAP). The exchange of commercial FM with more sustainable, recycled fish hydrolysates from aquaculture by-products additionally does not show any negative effect on the fish welfare. This supports the hypothesis that the alternative feed concepts presented here can replace the less sustainable commercial feeds.

4.5. Sensory Analysis

In the fish fed NOPAP^{+}, the fillet yield was higher, with a mean value of 41.2%, probably because NOPAP^{+} was enriched with Super Prime fishmeal and krill meal, which are known to produce better growth characteristics [34]. Interestingly, the alternative diets, PAP (39%) and NOPAP (38%), indicate no significant differences to the control-diet-fed fish (38%), resulting in the conclusion that the alternative formulation concepts do not negatively affect the fillet yield.

Sensory attributes are the most crucial factor for consumers, and the results of this study indicate that the control diet can be replaced by more sustainable alternatives (PAP and NOPAP) without affecting sensory attributes. According to the literature, softness, juice separation, taste, and smell are the most relevant attributes for consumers [51]. In this study, those attributes were not affected by alternative-diet-fed fish, in accordance with the recent literature for seabass (*D. labrax*) [50,52], greater amberjack (*Seriola dumerili*) [41], and gilthead seabream (*Sparus aurata*) [53]. The health benefits of fish from recirculation systems are apparent. This study shows that the values for fatty acids and the low toxic elements could encourage consumers to buy more fish. In addition, this health advantage of fish products does not fall in favor of a negative correlation between taste or consistency. This can be seen as a success for the effort to develop alternative and more sustainable feed concepts without losing sensory attributes. From an ecological point of view, the introduction of sustainable feeds is essential, but the economic difference between feeds should also be considered.

The most crucial factor in selecting new feed is to calculate the costs right from the beginning to the farm. This study tested four different feed concepts that could potentially replace the commercial feed for seabass. However, the raw materials used here were selected on an experimental basis, making estimating costs impossible due to excessive imprecision. By-products from the circular economy and artificially enriched algae were utilized for the sustainable ingredients in our diets. These are currently only used on a scientific basis and not commercially so that no realistic cost assumptions can be calculated. For the future, it would, therefore, be essential to make the by-products (such as fish hydrolysates from aquacultures) as well as enriched algae for fish feed available for commercial use.

5. Conclusions

This study shows that alternative diets using emerging and novel plant and animal-derived protein sources (both terrestrial and aquaculture by-products) have comparable performance to current commercial seabass feeds while being more sustainable. The fish welfare and sensory quality, which seem to be the strongest drivers for consumer appreciation, were not negatively affected by more sustainable feed concepts in which FM, FO, and soy products were replaced by several alternative ingredients (NOPAP and PAP). Furthermore, the PAP and NOPAP diets additionally had these traditional ingredients replaced by agro-industry, fisheries, and aquaculture by-products, to get into the direction of circular economies. The usage of aquaculture by-products in comparison to commercial FM has far less transportation distances (as it is usually reused at the same site) and additionally recycles the resources at hand, which make the overall production more eco-friendly. Therefore, this study provides evidence that alternative diets can successfully replace commercial diets for European seabass. The transfer to more sustainable diets would positively affect the consumers' attitude towards farmed fish (better sustainability with the same welfare and sensory status) and the fish farmers' potential for further sustainable and circular-economy-driven industrial growth. The recommendation for future feed formulations for farmed European seabass would be to replace the FM completely with hydrolysates, exchange the SBM with more sustainable plants, such as wheat and enriched algae, and add agriculture by-products, such as feather meal, etc., for a better mineral composition and digestibility.

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