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Nutrient Profile of Baltic Coastal Red Algae (*Delesseria sanguinea*), Baltic Blue Mussel (*Mytilus* spp.) and King Ragworm (*Alitta virens*) as Potential Feed Material in the Diet of Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792): A Preliminary Assessment

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Citation: Thum, G.; Cappai, M.G.; Bochert, R.; Schubert, H.; Wolf, P. Nutrient Profile of Baltic Coastal Red Algae (*Delesseria sanguinea*), Baltic Blue Mussel (*Mytilus* spp.) and King Ragworm (*Alitta virens*) as Potential Feed Material in the Diet of Rainbow Trout (*Oncorhynchus mykiss* Walbaum, 1792): A Preliminary Assessment. *Agriculture* **2022**, *12*, 196. https://doi.org/10.3390/agriculture12020196

Academic Editors: Vito Laudadio and Vincenzo Tufarelli

Received: 13 January 2022
Accepted: 25 January 2022
Published: 31 January 2022

Abstract: The use of selected Baltic coastal organisms as potential alternative feed material in the formulation of rainbow trout diets was studied. German coastal water organisms (*Delesseria sanguinea*, seaweed red algae (A); *Mytilus* spp., Baltic blue mussel (M); *Alitta virens*, king ragworm (W)) were analyzed for nutrient, amino acid and mineral composition, and tested in comparative feeding trials. Five dietary treatments were supplied to a total of 165 juvenile rainbow trout (778 ± 111 g) for 75 days, allotted in 15 special brackish (3–5 practical salinity units (PSU)) water basins consisting of 11 fish each (3 fish tanks (300 L) at 12 °C per feeding group). The fish were fed as follows: C group, 100% basic diet (control); A group, 10% red algae in C diet; M group, 10% mussel in C diet; W group, 35% ragworm in C diet; AW group, 10% algae + 30% ragworm in C diet. Feed provision was performed manually, once a day, with the feed offer adjusted to 1.8% of fish weight for the respective tank. The fish weight gain (WG) and feed conversion ratio (FCR) were recorded. In the proximate analysis of the different coastal organisms, the lowest crude protein content in dry matter (DM) was found in blue mussels (10.9%), whereas it was almost doubled in algae (21.8%), with the highest being found in the ragworm (63.1%). By contrast, the crude ash content was the highest in the mussel (84.4%, mostly due to $CaCO_3$ from the shell), much less in the red algae (28.1%) and the lowest in the ragworms (20.1%). The gross energy (GE) concentration was the highest in the ragworm (18.8 MJ × kg^{-1}), 12.1 MJ × kg^{-1} in the algae and the lowest in the blue mussel (2.93 MJ × kg^{-1}). The final weight of the fish ranged between 1780 and 2310 g at the end of the feeding trial, being the lowest for the fish fed the diet combined with red algae (A diet group) and the highest for the fish fed the control diet. No differences in FCR were found for the fish fed the five dietary treatments ($p > 0.05$), except for the W diet group (king ragworm has a lower FCR than that of the A group red algae, $p < 0.05$). The results from this trial suggest that at the tested amounts, both king ragworm and blue mussels are promising alternative feed material for rationing the rainbow trout diet, but not red algae, unless combined with ragworms.

Keywords: alternative protein source; amino acids; marine resources; rainbow trout

1. Introduction

The rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) farming system is one of the most important branches of onshore aquaculture. The flesh of rainbow trout is characterized by a high nutritional value and quality, for which the consumer's demand shows a continuous positive trend on the market [1,2]. The rainbow trout originates from rivers of the temperate and subpolar northern Pacific coast, and is known for its relatively high stress tolerance [3] and good feed conversion when raised in captivity. Nevertheless, as a carnivorous species, it shows relatively high protein demand in the diet. In this context, the open discussion about the need to look for alternative feed material for livestock farming and fishery fosters the scientific community to find viable and accessible solutions. The strategies of circular economy account a reduction in global food waste [4–6], with the aim of identifying non-conventional feeding sources for safe and sustainable animal feeding, non-competitive with humans [7,8]. The gap of dietary protein in animal feeding is mainly compensated by soy imports from South America. Soy and fish meal are two of the main components of carnivorous fish feeds in aquaculture. While soy needs to be imported, fish meal is getting rarer and more expensive, due to over fishing. Therefore, local and sustainable feed material might be a solution to maintain aquaculture nutrition that is less dependent on the global market, whilst also promoting local farming and feeding practices. In particular, the request for alternative protein sources in aquaculture has been ongoing for decades [9,10]. The literature reports different studies on the use of vegetal protein sources [11], but such ingredients often need the addition of amino acids and mineral supplementation to ensure sustainable production performance. New promising perspectives seem to originate from insect feeding in aquaculture, though the technological aspects of ingredient processing and the high costs still limit its widespread use at present [12]. In light of previous experiences, three alternative feed materials were identified to fit the purpose of responding to rainbow trout feeding. In particular, locally available marine coastal organisms, namely, red algae (*Delesseriasanguinea* (Hudson), J.V. Lamouroux, 1813), Baltic blue mussel (*Mytilus* spp.) and king ragworm (*Alitta virens*, M. Sars, 1835), appeared worthy of being investigated in detail. Thus, following a multitrophic aquaculture approach in the Baltic Sea, the nutritional assessment of local feeding sources may provide a new concept for advanced aquaculture feeding within the local ecological context. Moreover, these species are commercially produced at present, or there has been at least a first attempt for future production, to ensure a continuous and sustainable supply. Against this background, the rationale behind this investigation focused on the chance offered by such locally available natural feeds, and aimed to assess (a) the nutritional potentials of those marine coastal organisms as alternative feed material in dietary formulations and (b) to test the response of juvenile rainbow trout to different dietary treatments by a comparative feeding trial based on combination diets in comparison with a conventional control diet.

2. Materials and Methods

2.1. Feed Assessment

Sampling of native organisms from marine coasts of the Baltic Sea took place in the summer of 2014. Baltic blue mussel (*Mytilus* spp.) and seaweed red algae (*Delesseria sanguinea*) were harvested from the artificial reef Nienhagen. King ragworms (*Alitta virens*) were instead purchased from a commercial farm in the Netherlands (Topsy Baits) as frozen blocks.

The nutritional profile of such organisms was assessed by proximate analysis of the main nutrients, according to VDLUFA methods [13].

Analysis of the amino acid composition of protein was carried out by high-pressure liquid chromatography (HPLC), following the methods described by [14]. Macro- and micro-mineral analyses were carried out by ICP–mass spectrometry at the University of Hannover, by a collaborating working group.

2.2. Diets, Fish and Experimental Feeding

Based on the analytical data of the tested organisms, four experimental feeds were further developed. All experimental dietary treatments were based on commercial high-performance complete diet (BayWa Aqua Profi 4 mm, BayWa AG, München, Germany), which was used as control diet. The dietary treatments were formulated and developed in collaboration with the State Research Institute Mecklenburg-Vorpommern (LFA-MV). In particular, experimental diets were designed on the basis of the control feed, in which the combination of either red algae (A diet, 10% *Delesseria sanguinea*), Baltic blue mussel (M diet, 10% *Mytilus* spp.) or king ragworm (W diet, 35% *Alitta virens*), as well as a combination of red algae and king ragworm (A+W diet, 10% algae and 30% king ragworm), were obtained. Fish meal and linseed oil were added to ensure an isonitrogenous and isocaloric composition of the diets. The feeding trial was carried out in recirculating cross-connected fish tanks (300 L at 12 °C) at the LFA facilities in Born. In total, 165 juvenile rainbow trout were used. Fish were divided into 15 groups with 11 fishes per tank. All tested diets (including the control diet) were offered to three groups each; therefore, every dietary treatment was carried out for 3 replicate tanks per dietary treatment. Feed provision was performed manually, once a day, with the feed offer adjusted to 1.8% of fish weight for the respective tank.

During the feeding trial, fish were kept in efficient aquaculture conditions in respect to welfare of animals. All procedures adopted in the feeding trial ensured minimal animal manipulation and followed standard internal protocols, limited to feeding and weighing, in alignment with the animal welfare Directive 2010/63/EU. In particular, the quality of water was maintained through continuous disinfection and purification by tanks equipped with drum filter, moving bed bio-filter and UV light lamps. Additionally, oxygenation of water was assured to keep dissolved oxygen above 8 mg/L water, with weekly pH (safe range between 6.5 and 8) and temperature monitoring (12 °C).

2.3. Analysis of Data and Statistics

Data collected during experimental feeding were analyzed with one-way ANOVA, post hoc Tukey's test for grouping, with the diet (five levels, C = control diet; A = *D. sanguinea* combined diet; M = *Mytilus* spp. combined diet; W = *A. virens* combined diet; AW = *A. virens* + *D. sanguinea* combined diet) as fixed factor and fish performance as dependent variable. Statistical significance was set as p-value < 0.05.

3. Results

3.1. Nutritional Profile of Baltic Coastal Marine Feeding Sources

The crude composition of the marine organisms turned out to be heterogeneous, as expected. The nutrient content is reported in Table 1. The lowest crude protein content was found in the blue mussels (10.9%), whereas it was almost doubled in the algae (21.8%), with the highest being found in the ragworm (63.1%). By contrast, the crude ash content was the highest in the mussels (84.4%, mostly due to CaCO3 from the shell), much less in the red algae (28.1%) and the lowest in the ragworm (20.1%). The gross energy content (GE) (Table 1) correlates negatively to the crude ash values, and was the highest in the ragworm (18.8 MJ $\times$ kg^{-1}), 12.1 MJ $\times$ kg^{-1} in the algae and the lowest in the blue mussel (2.93 MJ $\times$ kg^{-1}).

Based on this data test, the feeds were formulated as shown in Table 2. In the algae and mussel test feed, fish meal had to be added. The goal to obtain isonitrogenous (crude protein content 44.3 $\pm$ 2.25 as% of DM) and isocaloric (GE content 23.5 $\pm$ 0.88 MJ $\times$ kg^{-1}) test feeds was accomplished within acceptable ranges. Important muscle protein building amino acids was similarly distributed in the crude protein of the feeds.

Table 1. Chemical composition of analyzed macronutrients, minerals and selected amino acids of tested organisms used as feed ingredients.

	D. sanguinea	***Mytilus* spp.**	***A. virens***
Nutrients (% DM)			
Crude ash	28.1	84.4	20.1
HCl insoluble ash	1.70	3.80	3.20

Table 1. *Cont.*

	D. sanguinea	*Mytilus* spp.	*A. virens*
Crude protein	21.8	10.9	63.1
Crude fat	<1.00	<1.00	7.62
Crude fibre	5.45	2.40	n.a.
N-free extract	43.7	1.30	9.18
Gross Energy (GE MJ $\times$ kg^{-1})	12.1	2.93	18.8
Minerals (g $\times$ kg^{-1})			
Ca	47.5	299	3.29
P	2.41	2.09	7.82
Cl	57.0	11.1	63.7
Na	33.5	8.47	38.0
K	42.7	2.33	15.3
Mg	8.20	1.96	5.41
Fe	1.66	0.04	0.54
Mn	2.91	0.01	0.01
Zn	0.16	0.02	0.11
Cu	0.02	0.004	0.01
Amino acids (g$*$100 g^{-1}) protein			
THR	4.34	3.58	3.43
LYS	4.62	4.98	5.64
ARG	5.92	5.64	5.90
CYS	2.29	1.49	1.00
MET	1.42	2.00	1.74
TRP	0.70	0.94	0.70

Table 2. Formulation of experimental diets, analytical nutrient composition and gross energy content (GE MJ $\times$ kg^{-1}).

	Diets				
	Diet C	**Diet A**	**Diet M**	**Diet W**	**Diet AW**
Items	Control	*D. sanguinea*	*Mytilus* spp.	*A. virens*	*D. sanguinea* + *A. virens*
Ingredients					
Complete diet	100	45.0	45.0	45.0	40.0
Blue mussel	0.00	0.00	10.0	0.00	0.00
King rag worm	0.00	0.00	0.00	35.0	30.0
Red alga	0.00	10.0	0.00	0.00	10.0
Fish meal	0.00	25.0	25.0	0.00	0.00
Linoil	0.00	15.0	15.0	15.0	15.0
Gluten	0.00	5.00	5.00	5.00	5.00
Nutrients					
crude ash	8.45	12.2	18.4	8.20	9.55
crude protein	46.7	43.3	42.1	46.9	42.9
crude fat	23.2	28.0	27.3	18.7	21.9
crude fibre	4.16	2.54	3.17	3.53	3.80
N-free extract	17.5	14.0	9.03	22.7	21.9
GE (MJ $\times$ kg^{-1})	17.5	14.0	9.03	22.7	21.9
Amino acids					
THR	3.49	3.35	3.36	3.48	3.38
LYS	6.14	5.41	5.51	5.73	5.38
ARG	5.52	5.44	5.55	5.40	5.19
CYS	1.00	1.01	0.99	0.98	0.94
MET	1.86	1.68	1.74	1.95	1.81
TRP	0.77	0.82	0.77	0.71	0.78

3.2. Performance of Rainbow Trout Fed the Experimental Diets

During experimental feeding, rainbow trout from all the groups displayed doubled average gains within 75 d from the start of the experiment. Only the A diet showed a trend of minor mass development compared to the C diet group. The growth performance per fish across the 75 d of experimental feeding is reported in Figure 1.

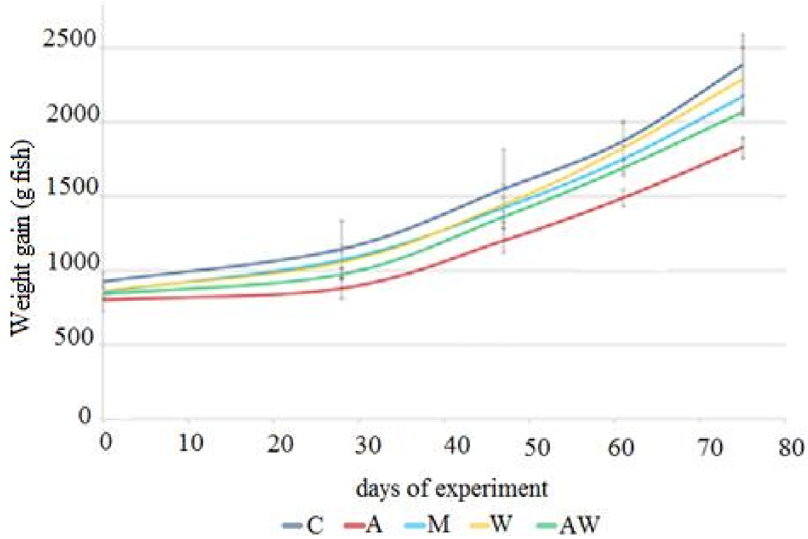

Figure 1. Trends of average gains of rainbow trout fed the 5 experimental diets across the feeding trial. A, *Delesseria sanguinea* combined diet; M, *Mytilus* spp. combined diet; W, *Alitta virens* combined diet; AW, *Alitta virens* + *Delesseria sanguinea* combined diet.

The fish in the C diet group (control) had a moderately higher final weight, with an average daily gain (ADG) of 0.018 g/d. The lowest ADG (0.014 g/d) was recorded for fish fed the red algae, *Delesseria sanguinea*, combined diet (A diet group), with the average final body weight being 23.9% lower than that achieved for fish fed the control diet (C diet group). However, no significant differences were found in regards to weight gain among the experimental groups. The best growth performance, based on data recorded for the combined experimental diets, was observed for fish fed the W diet (king ragworms, *Alitta virens*, ADG 0.018 g/d), followed by fish fed the blue mussel combined diet (M diet, ADG 0.018 g/d), in light of the FCR. The FCRs of all the test feeds are shown in Figure 2.

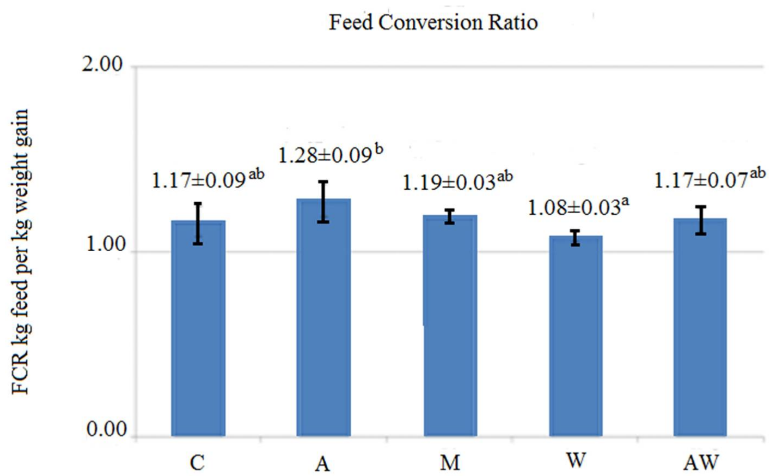

Figure 2. Feed conversion ratio (FCR) of rainbow trout fed the five experimental diets (C, control diet; A, *Delesseria sanguinea* combined diet; M, *Mytilus* spp. combined diet; W, *Alitta virens* combined diet; AW, *Alitta virens* + *Delesseria sanguinea* combined diet. Average values and SD are reported. Superscitps with different letters indicate statistical significance, $p < 0.05$.

Significantly, the FCR differed when king ragworm or red algae were combined in the diet, with the former showing the most favorable FCR. The combination of ragworms (35%) in the diet of rainbow trout produced the lowest FCR when compared to the other experimental diets, in a non-significant way, except for the A diet ($p < 0.05$).

4. Discussion

This investigation assessed, for the first time, the nutrient composition and potential combination of local marine coastal organisms in the diet of rainbow trout. Sufficient protein content and composition in commercially farmed ragworms *Alitta virens*, also sufficient phosphorous content of 7.82 g $\times$ kg^{-1} DM (6 g $\times$ kg^{-1} DM minimum recommended [15]), displayed to possess the highest potential for fishmeal supplementation; red algae, *Delesseria sanguinea*, which is relatively rich in protein, but also has a high N-free extract (NfE) fraction, mostly contained floridean starch [16], a typical energy storage molecule of red algae species, which may lead to digestive disorders; whole blue mussel, *Mytilus* spp., displayed an adequate protein composition, but low concentration, as well as high crude ash fraction due to the shell. Additionally, the $CaCO_3$ of the shell might also be a problem due to the potential compromising effects of acidic milieu in the stomach. Nevertheless, the blue mussel, *Mytilus edulis,* was found to be utilizable in trout feeds previously [17], but the performance is supposed to decline rapidly when trout are fed over 45% of the diet.

HCl insoluble ash, which can be interpreted as the sand fraction, was less than 4% in all the samples. Appreciable crude fat values were only found in the ragworm (7.62%). The NfE fraction was the highest in the red algae (43.7%), relatively low in the ragworms (9.18%) and the lowest in the mussel (1.3%). As mentioned before, the shell represents a large fraction of mussel meal, and this is the reason why noticeable amounts of Ca could be found in it (299 g $\times$ kg^{-1}); the ragworm meal showed the highest P content (7.82 g $\times$ kg^{-1}). The other organisms had *p* values less than 1/3 of the ragworm content. Noteworthy, manganese is the highest trace element found in the red algae meal (2.91 g $\times$ kg^{-1}). The amino acid composition of the tested organisms, as an alternative source of protein, did not allow growth differences to be identified in the patterns observed between the feeding groups.

All the tested groups showed comparable weight gain throughout the experimental feeding period, except for fish fed the red algae diet (A diet group), who showed comparatively lower weight gain. This finding is also emphasized by evidence from the literature, in which red algae species used as ingredients in dietary formulations showed limited weight gain [18–20]. Despite the fact that marine seaweeds, including red algae, may be valuable sources of carotenoids, which are of importance for fish and aquatic birds, analogues of terrestrial plants for mammals in the natural environment [21], the performance of fish fed *Delesseria sanguinea* in the combined diet was unsatisfactory at the tested amount. Except for the A diet group, the rest of the groups fed the different experimental diets displayed comparable weight gain to fish fed the control diet. All the tested diets showed no significant differences in FCR when compared to fish of the control diet group. Noteworthy, the lowest FCR was found with the ragworm-only combination (significant difference with red algae). Moreover, the king ragworm combination was also used to improve the performance of fish fed red algae in the diet. In fact, when 30% ragworm meal and 30% plant-based ingredients (algae meal, linseed oil and gluten) were used, the fish performance improved. In this perspective, the potential use of red algae and ragworms in the diet of rainbow trout may pave the way for further investigations to optimize the most appropriate combination amount for different quality traits (color and stability, for instance) of flesh from farmed fish fed local ingredients.

5. Conclusions

The nutrient profile of alternative Baltic coastal feed for rainbow trout diets is reported here for the first time. Such feeding sources were tested in different feeding trials, alone or in combination, and compared with a conventional control diet for rainbow trout. The results from this experimental trial appear to be suggestive of the promising use of king ragworms or blue mussels as ingredients combined in the diet for rainbow trout. The fact that king ragworms and blue mussels represent locally available alternative feed material is advantageous for the potential of meeting the request of a sustainable and environmentally friendly production chain. Red algae led to unsatisfactory growth performance at the substitution level tested in this investigation, except when combined with king ragworms in the diet.

Author Contributions: Conceptualization, G.T., P.W. and R.B.; methodology, G.T., P.W., R.B., M.G.C. and H.S.; software, G.T.; validation, G.T., P.W. and R.B.; formal analysis, H.S.; investigation, G.T., R.B., P.W., M.G.C. and H.S.; resources, P.W., R.B. and H.S.; data curation, G.T.; writing—original draft preparation, G.T.; writing—review and editing, all authors; visualization, all authors; supervision, P.W., R.B. and H.S.; project administration, R.B.; funding acquisition, R.B. and P.W. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Ethical review and approval were waived for this study, due to farming practices only.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data of results reported in this study are available on request to the corresponding author. The data are not publicly available due to the fact that all essential information of the article appear in tables and figures of the paper.

Conflicts of Interest: The authors declare no conflict of interest.

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