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Effect of *Calanus finmarchicus* Hydrolysate Inclusion on Diet Attractiveness for Whiteleg Shrimp (*Litopenaeus vannamei*)

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Abstract: Shrimp feed formulations have moved towards less fish meal and more of the readily available and cheaper plant proteins. To counteract the lower attractiveness and palatability of plant proteins, feeds are supplemented with ingredients known to have chemoattractive properties that will increase feed intake. This study investigated the putative chemoattractive effect of *Calanus finmarchicus* hydrolysate, when used as a dietary supplement in shrimp feeds. *C. finmarchicus* is a zooplankton species native to the northern Atlantic Ocean and is a novel and sustainable raw material for shrimp feed products. Diet attractiveness was evaluated in a 24-day feeding trial with whiteleg shrimp (*Litopenaeus vannamei*) by measuring the intake of 12 diets with various levels of fish meal, calanus hydrolysate, and krill (*Euphausia superba*) meal. Higher inclusion rates of both ingredients resulted in increased feed intake, and supplementing the high fish meal diet with calanus hydrolysate gave a statistically significant higher feed intake. Low molecular weight peptides, chemoattractive amino acids, and the water-soluble nature of the hydrolysate could explain the chemoattractive properties observed in the study.

Keywords: chemoattraction; diet attractiveness; calanus; feeding trial; fish meal; krill; whiteleg shrimp; *Litopenaeus vannamei*

Key Contribution: A novel protein hydrolysate from the zooplankton species *Calanus finmarchicus* can increase the attractiveness of diets for whiteleg shrimp (*Litopenaeus vannamei*).

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1. Introduction

Shrimp and prawns are sources of lean protein and other nutrients beneficial for human health [1]. Whiteleg shrimp (*Litopenaeus vannamei*) is also of great economic importance and was the most widely produced aquaculture species globally in 2020, with a total production volume of 5.8 million tonnes [2]. The amount of feed necessary to farm whiteleg shrimp is much higher than the volume of marine ingredients currently available. In recent years, shrimp feed formulations comprise less fish meal and more readily available and cheaper plant proteins [3]. However, a general concern with using mainly plant ingredients in aquaculture diets is the apparent lack of attractiveness and palatability, and it has been especially hard to replace fish meal for carnivorous fish and crustaceans [4]. Diets with high levels of plant proteins have been linked to reduced feed intake and poor growth performance for whiteleg shrimp [5]. In addition to the obvious economic perspectives of reduced growth and unutilized feed, the accumulation of excess feed at the bottom of the shrimp ponds has detrimental environmental effects [6].

Shrimp are chemosensory feeders, using systems of olfaction and chemoreception when acquiring and consuming food [7]. The inclusion of attractants that activate the chemosensory systems is common to increase the attractiveness of diets with high plant protein content [8]. Natural chemoattractants for shrimp are often marine ingredients rich in free amino acids, nucleotides, nucleosides, quaternary ammonium compounds, phospholipids, and biogenic amines, which are recognized by the chemosensory system of aquatic animals when locating and ingesting feed materials [9,10]. In addition, dietary inclusion of marine ingredients in water-soluble form (e.g., protein hydrolysates) has been found especially attractive and palatable [11,12].

Zooplankton serve as natural food sources for wild shrimp as they grow from omnivore larvae to the more carnivorous post-larval stages. Antarctic krill (*Euphausia superba*) is a zooplankton species already in use for shrimp diets. Krill meal inclusion in diets for whiteleg shrimp has been shown to increase feed intake [13,14] and improve overall nutritional quality [15–17].

A novel resource of zooplankton products with potential for feed applications is *Calanus finmarchicus*, a species in the northern Atlantic Ocean with an annual biomass production estimated at 290 million tonnes [18,19]. The enormous production combined with a suitable nutrient composition make *C. finmarchicus* a significant and relevant resource for both human consumption and animal nutrition [20–22]. One of the novel products made from *C. finmarchicus* is a low molecular weight protein hydrolysate, found to increase fish growth in a recently published article [23].

The primary objective of this research is to rigorously evaluate the potential chemoattractive effects of *C. finmarchicus* hydrolysate as a supplement in shrimp feed formulations. The study aims to quantify the impact of this novel zooplankton-derived ingredient on the feeding behavior and intake levels of whiteleg shrimp. The ultimate goal is to identify an effective strategy to enhance the palatability of plant protein-rich diets and thereby optimize the growth performance and sustainability of shrimp aquaculture practices.

2. Materials and Methods

2.1. Diet Formulation and Production

Calanus finmarchicus was harvested and processed into calanus hydrolysate (CH) by Calanus AS (Tromsø, Norway), and the krill meal (KM) was purchased from a commercial producer. The nutritional composition of CH (liquid) and KM (powder) is presented in Table 1.

The experimental diets were formulated as isonitrogenous (38% crude protein), isolipidic (7% crude lipids), and isoenergetic (17.6 MJ/Kg). Inclusion levels of certain ingredients were adjusted to ensure equivalent levels of nutrients in the diets (Table 2). Twelve diets were produced, of which half received a low fish meal content (10%), and the other half had a high fish meal content (20%). For each of the six diets in the two groups, one control diet was without test ingredients (compensated by wheat gluten meal), three were with varying concentrations of CH (2%, 4%, 6%), and two were with varying concentrations of krill meal (2%, 3%). Ingredient inclusion rates were chosen on the basis of the different dry matter contents of CH (53.3%) and KM (91.6%).

Diets were produced at Matís Aquaculture Research Station, where crumbled pellets were made from raw materials. All dried ingredients were ground thinly using an MF-300 automatic mill grinder (Ipharmachine, Ruian, China). Raw materials were mixed thoroughly using an AE200 commercial mixer (Hobart, London, UK), while oil and liquid ingredients were poured into the mixture. Finally, water was slowly added to obtain a paste texture, which was processed into strings through an FL82 (ADE, Hamburg, Germany). The strings were spread on trays and dried for 48 h at different temperature levels from 70 °C to 30 °C using an OTS4 drying cabinet (Kreuzmayr, Wallern an der Trattnach, Austria). Dried feeds were pelleted using the grinder to a size of 2 mm and stored in closed buckets at room temperature in a dry and ventilated area. All the diets were submitted to the Matís lab (Reykjavik, Iceland) for analysis of crude protein (ISO, 16634-1:2008) [24], crude fat (AOCS Ba 3-38) [25], ash (ISO 5984:2002) [26] and moisture (ISO 6496:1999) [27]. Table 3 shows the analyses of the proximates in the diets.

Table 1. Nutritional composition of the test ingredients.

Component	CH [1] (WW)	KM [2] (WW)	CH [3] (DW)	KM [3] (DW)
Dry matter, %	53.3	91.6	100.0	100.0
Crude protein, %	33.6	55.0	63.0	60.0
Crude fat, %	0.6	25.2	1.1	27.5
Ash, %	10.9	8.6	20.5	9.3
Phosphorus, %	0.8	1.2	1.5	1.3
Gross energy, kJ/g	9.8	24.7	18.4	27.0
Arginine, %	2.23	3.39	4.18	3.65
Histidine, %	0.46	1.24	0.86	1.43
Isoleucine, %	1.21	2.87	2.27	2.98
Leucine, %	2.07	4.42	3.88	4.61
Lysine, %	2.26	4.03	4.24	4.21
Threonine, %	1.23	2.43	2.31	2.60
Valine, %	1.58	2.91	2.96	3.09
Methionine, %	0.62	2.00	1.16	1.68
Cysteine, %	0.33	0.43	0.62	0.45
Phenylalanine, %	1.08	2.93	2.03	3.17
Tyrosine, %	1.19	3.25	2.23	3.55
Aspartic acid, %	2.56	5.73	4.80	6.26
Glutamic acid, %	3.85	7.05	7.22	7.70
Alanine, %	2.12	2.99	3.98	3.26
Glycine, %	2.29	2.58	4.30	2.82
Proline, %	1.13	2.18	2.12	2.38
Serine, %	1.11	2.22	2.08	2.42
Taurine, %	0.56	0.14	1.05	0.15

CH: Calanus hydrolysate; KM: Krill meal; WW: Wet weight; DW: Dry weight. [1] Data from Bøgwald et al. [23]. [2] Data from Nunes et al. [14]. [3] Calculated from WW values.

Table 2. Formulation of the 12 experimental diets.

Ingredients (%)	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8	Diet 9	Diet 10	Diet 11	Diet 12
Fish meal [a]	10	10	10	10	10	10	20	20	20	20	20	20
Calanus hydrolysate [b]	0	2	4	6	0	0	0	2	4	6	0	0
Krill meal [c]	0	0	0	0	2	3	0	0	0	0	2	3
Fish oil, anchovy [d]	1.37	1.39	1.4	1.42	1.09	0.94	0.46	0.47	0.49	0.5	0.17	0.03
Wheat gluten meal [e]	11.54	10.77	10.01	9.24	9.87	9.04	10.56	9.81	9.07	8.32	8.92	8.1
Wheat grain, standard	6.36	6.22	6.07	5.93	6.54	6.64	16.97	16.72	16.46	16.21	17	17.01
Soybean meal [f]	25	25	25	25	25	25	16	16	16	16	16	16
Rice bran	15	15	15	15	15	15	14	14	14	14	14	14
Sunflower meal	20	20	20	20	20	20	12	12	12	12	12	12
Lecithin soya crude	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Diatomaceous earth	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Cholesterol, feed grade	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Carboxymethylcellulose	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Mono ammonium phosphate	2.73	2.68	2.62	2.57	2.63	2.59	2.43	2.38	2.33	2.27	2.34	2.29
Vitamins [g]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Minerals [h]	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine [i]	0.08	0.06	0.04	0.02	0.05	0.04						
L-Lysine HCL [i]	0.23	0.17	0.11	0.05	0.14	0.1						

[a] 68.5% crude protein, 999 (Denmark). [b] Calanus AS (Norway). [c] Commercial supplier (unspecified). [d] South America. [e] 78% crude protein. [f] 48% crude protein (Brazil). [g] Simplyfish aqua (Norway). [h] Simplyfish shrimp (Norway). [i] Inclusion in low fish meal diets to ensure equivalent levels.

Table 3. Proximate composition of experimental diets. Values are percentages of each diet.

Proximate (%)	Low Fish Meal						High Fish Meal					
	CTRL	CH2	CH4	CH6	KM2	KM3	CTRL	CH2	CH4	CH6	KM2	KM3
Moisture	7.6	7.3	6.7	6.9	5.8	5.2	6.3	6.4	6.8	7.7	7.6	7.6
Protein	41.11	40.4	41	40.82	41.13	41.24	39.87	40.17	39.59	39.62	39.3	39.24
Fat	6.6	6.9	6.7	6.7	6.6	6.8	6.5	6.7	6.6	6.5	6.4	6.5
Ash	10.3	10.6	10.7	10.7	10.6	10.8	10.9	11.1	11.2	10.9	10.9	11.2

CTRL: Control diet. CH2: Diet with 2% calanus hydrolysate. CH4: Diet with 4% calanus hydrolysate. CH6: Diet with 6% calanus hydrolysate. KM2: Diet with 2% krill meal. KM3: Diet with 3% krill meal.

2.2. Ethical Statement

Matís (Reykjavik, Iceland) is granted operating licenses (no. FE-1134) by the Icelandic Food and Veterinary Authority (MAST) with reference to section III of Act No. 71/2008 on fish farming, and the experimental protocol of the trial is covered by these licenses. Experiments were conducted by FELASA certified scientists, in full compliance with ARRIVE guidelines and the European (Directive 2010/63/EU) and Icelandic (Animal welfare law no. 55/2013) legislation on the protection of animals for scientific purposes.

2.3. Feeding Trial

The trial was performed at the Matís Aquaculture Research Station (MARS) (Reykjavik, Iceland), with whiteleg shrimp (*L. vannamei*) shipped from White Panther Produktion GmbH at the stage of 12 days post-larvae. Shrimp were grown for 3.5 months until small juveniles before the trial started, and 720 shrimp weighing 3.67 ± 0.01 g (weighed individually) were evenly distributed in 36 identical tanks at an initial stocking density of 20 individuals per tank. The trial was executed in one of the experimental units at MARS, with three Recirculating Aquaculture Systems (RAS), each fitted with 12 circular tanks (0.36 m^2 bottom area, 200 L) and a filtration area. The water was processed by a sponge for solid removal, a moving bed, a protein skimmer, and a UV light. Systems were fitted with a heater to keep the temperature level stable.

Husbandry conditions were kept as close to optimal as possible (Table S1 in Supplementary Data). Artificial sea water, obtained from mixing tap water with salt bags (Aqua Media), was used to fill up the tanks. The salinity level was kept at 25.2 ± 0.5‰ ppm. Temperature and pH were maintained at 30.1 ± 0.5 °C and 7.2 ± 0.1, optimal conditions for whiteleg shrimp according to Wyban et al. [28]. The pH was buffered on setpoint by a solution of sodium hydroxide dissolved in water at a 1:1000 ratio. Oxygen levels were maintained using a single air stone in each tank, close to saturation level (100%), which is around 6.75 ppm in regard to the temperature and salinity mentioned above. The light intensity and the photoperiod were maintained automatically, and the light regime followed a 12:12 light cycle. Daily water quality measurements were performed before the first feeding period. Oxygen saturation level, temperature, salinity, pH and the nitrogen effluents nitrite, nitrate, and ammonium were recorded.

The 12 experimental diets were fed 3 times per day for 24 days. Diets were switched every day from one tank to another, and every tank thus received each diet twice at the end of the trial. Feeding was at 8:30 am, 11:30 am, and 2:30 pm, and lasted for 1.5 h. All the diets were tested for water stability (leaching, dissolving) prior to the trial to ensure their integrity during the feeding periods. Diets were distributed using submersed feeding trays, and the feed amount administered each time was set according to the stocking density, mean body weight, and the intervals between feeding times. Uneaten feed was syphoned, collected, dried for 36 h at 60 °C, and weighed. Feed consumption was calculated by the mass difference between distributed and uneaten feed.

2.4. Statistics

Data assessment of feed attractiveness was based on the feed intake values per experimental diet. Data were recorded in dry matter per feeding day and converted to percentages relative to the negative control diet, with moisture content of all diets deducted (Table S3 in Supplementary Data). Statistical comparisons were made using a one-way analysis of variance (ANOVA) or a Kruskal–Wallis non-parametrical test, depending on the distribution of the data and variance equality (Tables S4 and S5). Feed intake was presented as mean ± standard deviation, with $n = 3$ replicate tanks for each diet. Simple linear regression was applied to the data to generate feed intake response curves based on ingredient inclusion rates (Tables S6 and S7). Statistical tests were performed using R studio, Inc (version 4.1.1), and graphs were made using GraphPad Prism (version 10.1.0).

3. Results and Discussion

The whiteleg shrimp fully accepted all the diets from the start (mean body weight 3.67 g) and throughout the experiment until the end (mean body weight 10.46 g). The daily rotation study design (Figure 1) of switching the diets to new tanks each day was also successful. This specific design excluded any potential tank bias in the study, but also meant that only collective performance parameters could be measured beyond feed intake. All the parameters were well within acceptable ranges; survival in the experiment was 99.6%, specific growth rate was 4.18%/day, and the feed conversion ratio was 1.76. The rest of the collective performance parameters can be found in Table S2 of the Supplementary Data.

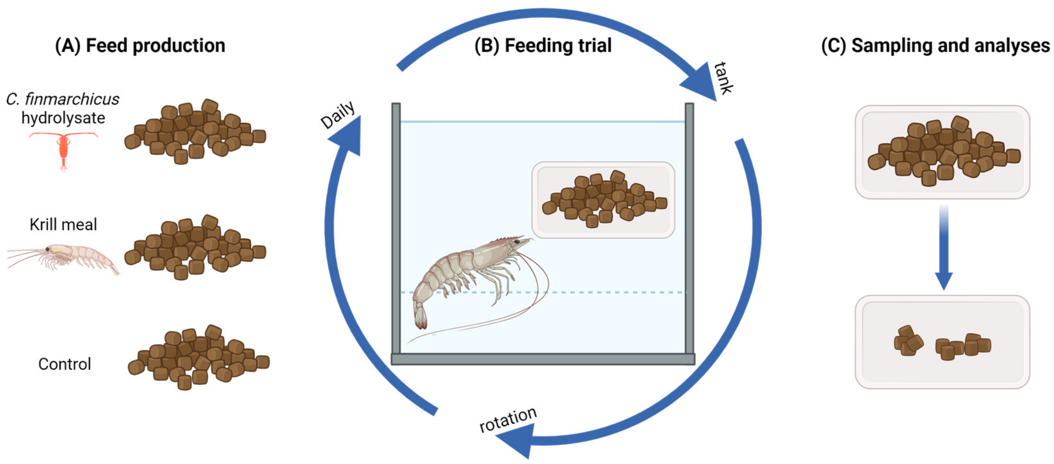

Figure 1. Study design overview of the attractiveness feeding trial with whiteleg shrimp (*Litopenaeus vannamei*), quantifying daily feed intake of 12 experimental diets (36 tanks, $n = 3$). (**A**) Diets with low and high fish meal were produced with different inclusion levels of an attractive ingredient (*Calanus finmarchicus* hydrolysate or krill meal). (**B**) Submerged feeding trays were given three times a day. Daily rotation of the 12 diets were performed to exclude tank bias, and every tank received each diet twice. (**C**) Uneaten feed was collected after each feeding time, and feed intake comparisons were made on the difference between distributed and uneaten feed for every diet. Figure created with BioRender.com.

3.1. Feed Intake in Low/High FM Groups

The overall feed intake results were similar for both low and high FM groups, wherein the intake of CH diets increased with higher inclusion rates, while diets with KM had an intermediate effect (Figure 2). However, only the results in the high FM group were statistically significant (Figure 2B). Unequal variances in the low FM group made proving statistical significance more difficult, as it demanded a non-parametrical test like Kruskal–Wallis instead of the standard ANOVA (Figure 2A, Table S4 in Supplementary Data). Diets with two different FM levels were tested to investigate if the attractive ingredients had especially pronounced effects in either low or high FM groups, formulated with either 10% FM to represent the level commercial feeds are currently moving towards, or 20% FM to represent the traditional level of shrimp feed. Chemoattractive ingredients are often included in commercial diets with mainly plant proteins, to counteract their lower attractiveness and palatability [10]. Considering that FM contains more chemoattractive substances than plant proteins, especially ATP/nucleotides and marine metabolites, feed intake should be higher in the high FM group and differences among diets should theoretically be larger in the low FM group. However, the intakes of low and high FM control diets were found to be equal (Table S3 in Supplementary Data), and the relatively moderate inclusion rates of attractive ingredients thus had a larger influence on feed intake. The effects of attractive ingredients are supposedly higher when used in combination rather than individually [9], which can help explain the increased differences among the high FM diets compared to the low FM group.

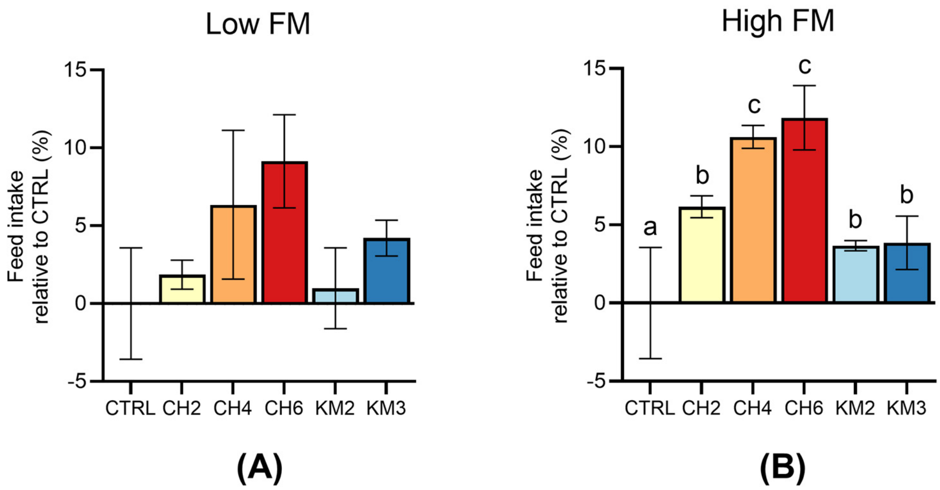

Figure 2. Feed intake of experimental diets with varying inclusion levels of an attractive ingredient (Calanus hydrolysate, CH or krill meal, KM), in percentage relative to control diets. (**A**) Diets formulated with low fish meal (FM, 10%) and an attractive ingredient. (**B**) Diets formulated with high FM (20%) and an attractive ingredient. CH2: Calanus hydrolysate 2% inclusion. CH4: Calanus hydrolysate 4% inclusion. CH6: Calanus hydrolysate 6% inclusion. CTRL: Control. FM: Fish meal. KM2: Krill meal 2% inclusion. KM3: Krill meal 3% inclusion. Bars are means ± standard deviation (n = 3). Lower-case letters (a, b, c) denote statistically significant differences between the diets ($p < 0.05$).

Processing raw materials with enzymatic hydrolysis yields protein hydrolysates, which are soluble protein fractions abundant in chemoattractive substances, such as free amino acids, nitrogen-containing marine metabolites, and peptides. Low molecular weight peptides and metabolites have been found to be especially important for feed attractiveness and palatability [29–31], and approximately 87% of the protein in CH are free amino acids and peptides < 1000 Da (Table S8 in Supplementary Data). The observed effect in this study could stem from a combination of the chemoattractive content and the water-soluble nature of CH, which allows leaching of the chemosensory compounds into the water surrounding the shrimp, leading to increased attractiveness and ultimately feed intake.

3.2. Feed Intake and CH/KM Inclusion Rates

Combined data from low and high FM groups show that the feed intake increased in line with the inclusion rates of both attractive ingredients (Figure 3). Marine proteins known to enhance feed attractiveness and palatability for shrimp, such as squid and krill, are included at low dietary levels, often between 0.5% and 5%, sufficient to elicit positive feeding behavioral responses without too heavy an impact on formulation costs [10,32].

KM was included at 2% and 3% in the diets of this study, mimicking its typical commercial inclusion rates and also according to the levels found optimal in several studies [14,31,33]. As this was the first study of the chemoattractive properties of CH, its inclusion rates were tested using an incremental increase from a low KM level (2%), via an intermediate level (4%) to the highest commercially relevant inclusion (6%) to find the most suitable level for shrimp feed. Although plotted on a limited set of data points, a comparison of the ingredients on an as-is basis (wet weight) showed that feed intake increased more for diets with CH than KM as inclusion rates increased (Figure 3A). Considering that CH is a liquid product and KM is a dry meal, a direct comparison was normalized to their dry weight, demonstrating an even more prominent effect of CH compared with KM (Figure 3B). If the inclusion rates had been pushed beyond what was tested in this experiment, the feed intake response would likely flatten out in the manner of dose–response curves. However, such high inclusion levels would not be considered commercially viable due to the cost vs. benefits of these marine ingredients in feed formulations.

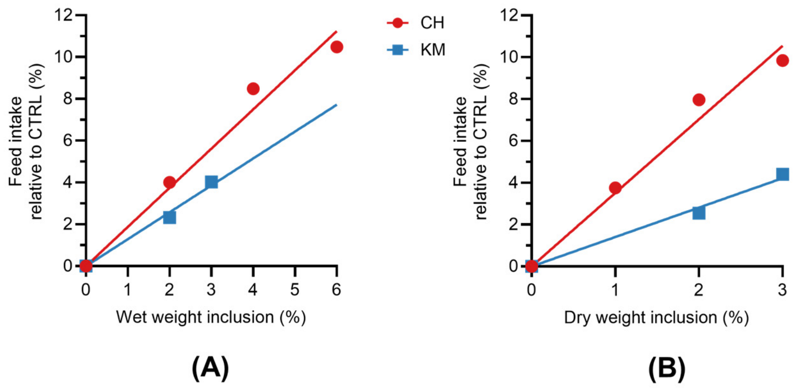

Figure 3. Feed intake response for attractive ingredient diets relative to negative control, using simple linear regression with dietary inclusion rate (%) as the single explanatory variable. Based on combined data from low and high fish meal groups (*n* = 3 for every diet in both groups). (**A**) Response based on inclusion rates (%) of the ingredients. (**B**) Response based on inclusion rates (%) of the dry matter content of the ingredients. CH: Calanus hydrolysate. CTRL: Control. KM: Krill meal.

Alkaline and neutral amino acids, such as glycine, proline, taurine, and valine, and derivatives, like betaine, are known to have chemoattractive properties for olfaction and/or ingestion in carnivorous species [9,10]. Looking for further answers as to why diets with CH showed higher attractiveness, a comparison of the chemoattractant amino acids in the attractive ingredients was made. The two most striking differences were found for glycine and taurine, both of which were notably higher for CH than KM on dry matter basis (Table 1). CH is especially rich in taurine, a neutral non-proteinogenic amino acid widely distributed in animal tissues, and the results of the study indicate that it is important for diet attractiveness. Plant proteins are generally deficient in taurine, possibly explaining part of their lower attractiveness and palatability [34]. In addition to increased feed intake, supplementation of taurine in diets for juvenile whiteleg shrimp has been found to improve growth performance, metabolism, immunity, anti-oxidant capacity, and intestinal health [35–39]. Metabolomic fingerprinting of *C. finmarchicus* has also revealed that one of its major metabolites is betaine [40], which is a highly soluble glycine derivative with chemoattractive properties for both fish and shrimp [8–10,41–43].

On a final note, this study shows that products of both Arctic and Antarctic species are perfectly suited as ingredients for increased attractiveness of diets for a tropical species of shrimp. Increased feed intake should also mean reduced feed waste, thereby improving the efficiency and sustainability of shrimp farming practices through reducing the amount of unutilized feed, ultimately leading to cleaner wastewater and less negative environmental impacts from the industry. Juveniles of whiteleg shrimp were used in this study, as most other comparable nutritional studies are carried out under controlled conditions at juvenile or post-larval stages. Chemoattractant properties and optimal nutritional composition of feeds are also especially important in early life stages, wherein low feed intake and sub-optimal growth can make shrimp more vulnerable to diseases and increase the risk of cannibalism. Considering that the study was designed specifically to measure feed intake, the daily rotation of feeds to new tanks excluded any potential tank bias but also prevented measuring any other criteria in the trial. As of now, there are no other published studies on the effects of products from *C. finmarchicus* in shrimp feeds, and the promising results on chemoattraction presented here should motivate further exploration of the nutritional and functional properties of products from this novel raw material for shrimp aquaculture.

4. Conclusions

The novel protein hydrolysate from *C. finmarchicus* increased attractiveness of formulated feeds for whiteleg shrimp in this study, wherein diets with CH inclusion showed higher feed intake compared with the negative control and diets with krill meal inclusion. Feed intake increased in line with higher inclusion rates, and the notably high chemoattractant properties of CH could be attributed to a combination of its water-soluble nature, low molecular weight peptides, and content of known chemoattractive amino acids. Future follow-up studies on the hydrolysate should focus on the nutritional and functional properties, to investigate how the ingredient also can affect shrimp growth, survival, and overall health.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9040134/s1, Table S1: Water parameters in the study; Table S2: Collective performance parameters of whiteleg shrimp fed the attractive diets for 24 days; Table S3: Feed intake and moisture contents for each diet, with values in grams (g); Table S4: Summary of Kruskal–Wallis non-parametrical test, for low fish meal group. Table S5: Summary of one-way ANOVA, for high fish meal group; Table S6: Simple linear regression (response curve), by ingredient wet weight inclusion; Table S7: Simple linear regression (response curve), by ingredient dry weight inclusion; Table S8: Molecular weight distribution of calanus hydrolysate (CH).

Author Contributions: I.B., S.H., A.M.P., S.G.W., and K.-E.E. contributed to the conception and design of the study. S.H. with staff at Matís performed the feeding trial and analyses. I.B. and S.H. wrote the first draft of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Matís (Reykjavik, Iceland) is granted operating licenses (no. FE-1134) by the Icelandic Food and Veterinary Authority (MAST) with reference to section III of Act No. 71/2008 on fish farming, and the experimental protocol of the trial is covered by these licenses.

Informed Consent Statement: Not applicable.

Data Availability Statement: All data is available in the article or the Supplementary Material.

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Conflicts of Interest: Authors Isak Bøgwald and Alice Marie Pedersen were employed by the company Calanus AS. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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