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Research Article

Effects of Feeding an All-Plant Diet on Rainbow Trout Performance and Solid Waste Characteristics

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Plant-based ingredients have become a main component of modern salmonid feeds, substituting for a significant proportion of fish-based ingredients while maintaining a high nutritional quality. But besides the nutritional value of a feed, there are other important aspects to be considered when altering formulations, foremost amongst which is the impact on water quality. A 100-day trial was conducted to evaluate the impact of different feed mixtures on fish performance, faecal stability, and particle size distribution. In a two-factorial experiment, three feed treatments (a fish-based reference, a diet with 10% hemoglobin meal, and a plant-based alternative) were trialled with and without inclusion of 0.3% dietary binder and were fed to triplicate groups of rainbow trout. While fish performance was not affected by dietary treatment, rheological measurements showed that the stability of faeces from fish consuming plant-based diets was distinctly reduced. Strong binder effects were observed for all dietary treatments, but faeces resulting from the pure plant-based diet produced more small particles and resulted in lower removal efficiencies by drum filter and sedimentation than those resulting from other dietary treatments. The implications of these findings for sustainable and effective aquaculture are discussed.

1. Introduction

Substitution of marine-based dietary components in feeds has been on the sustainability agenda of the aquaculture sector for decades [1]. Following the development of dry feeds in the 1950s, fish meal and fish oil from marine pelagic forage fish were core ingredients for almost all farmed carnivorous fish species for reasons including ready availability, high digestibility, palatability, and the quality and quantity of essential amino and fatty acids [2].

After the rapid growth of the aquaculture industry over the last four decades, serious questions have been raised about the heavy reliance of the sector on marine resources. Pressure on forage fish stocks led to sharp increases in fish meal and fish oil prices [3] and ethical questions about the intensive use of food grade forage fish stocks in animal feed [4]. The high ratio of fish-in to fish-out in farmed carnivorous species was of particular concern. The industry response was fairly prompt and, for the last 30 years, has seen the development of feeds for many species in which fish meal and fish oil are partly replaced with plant-based raw materials, primarily soy, wheat, and rapeseed [5]. The marine components of salmon feeds declined from 90% in 1990 to less than 25% in 2016 [6], and that of commercial rainbow trout dropped further still, suggesting that for some relevant carnivorous species at least, aquaculture is capable of achieving net production of fish. While experiments involving the complete substitution of fish content (including the oil fraction) by plant-based ingredients in rainbow trout diets suggest that fish performance might be kept at acceptable levels [7], such a move does present other challenges, such as impacts to fish health caused by antinutritive substances in grain- or oilseed-based protein sources which are costly to overcome

[8]. Another major issue is the loss of omega-3 fatty acids DHA and EPA from fish reared on vegetable oils, reducing the human health benefits of fish consumption [9].

Furthermore, while generally considered sustainable at first glance, the substitution of fish meal can also have consequences that undermine sustainability, in particular in terms of faecal consistency. That feed composition which affects faecal stability is obvious, and scientific studies clearly show that higher levels of plant protein in salmonid diets result in greater faecal fragmentation [10, 11] and increased production of fines, which are environmentally problematic and provide particular challenges in the management of land-based aquaculture operations [12].

One effective and proven way to favourably modify faecal consistency to counteract the breakdown of solids is the targeted incorporation of binder additives such as guar gum. Previous studies have revealed that even minimal levels of dietary guar gum yield strong effects on faecal stability, with positive consequences for water quality due to enhanced potential for solid removal. As a result, this approach is now widely used in salmonid feeds for RAS around the world [13] and increasingly for other species. However, this effect is mostly lost in feeds comprising predominantly plant raw material, as data from Brinker and Friedrich [10] indicates.

Stabilising effects can be contributed by individual ingredients added primarily to their nutritive function. Blood meal is one example. When appropriately processed, this relatively cost-efficient low phosphorous ingredient also combines valuable protein content with excellent digestibility [14]. After a ban in the European Union following the BSE crisis, nonruminant blood products were reauthorized for use in fish feed in 2003, and today, blood or hemoglobin meals from swine or poultry are optional ingredients for commercial salmonid feeds.

The current study addresses the fish performance and system waste load consequences of partial and complete substitution of marine ingredients by plant alternatives in rainbow trout diets. Three dietary treatments were formulated: a control with fish meal and fish oil as the main ingredients; a partial replacement treatment containing a mixture of hemoglobin meal and plant-based proteins, fish oil, and rapeseed oil; and a third treatment formulated exclusively from raw materials of plant origin.

In order to compensate for anticipated negative effects of plant-based raw material on the mechanical properties of faeces, secondary formulations of each treatment were created with 0.3% of guar gum level, a non-starch polysaccharide binder (NSP) which has previously proved effective in stabilizing faecal matter of rainbow trout [13].

2. Materials and Methods

2.1. Diets and Husbandry. In a two-factorial feeding trial, six extruded iso-energetic and iso-carbohydrate experimental diets were each tested on triplicate groups of rainbow trout *Oncorhynchus mykiss* (Störk strain), with 40 fish per replicate tank. The three protein treatments comprised a fishmeal-based diet (FB), a diet with 10% hemoglobin meal (HEM), and a plant-based (PB) diet; each of which was trailed with and without the addition of 0.3% guar gum. All diets also contained 0.02% of the inert, indigestible marker yttrium oxide (Y_2O_3) to allow for digestibility measurements [15, 16]. Detailed composition data is given in Table 1.

All diets were tested on randomly allocated triplicate groups of 40 rainbow trout with an initial body weight of 71 ± 11 g (mean ± S.D.). The fish were fed six days a week (Sunday to Friday) with a daily allowance of 1.2% body weight.

Approximately 40% of the daily ration was dispensed manually between 08:00 h and 08:40 h, with continuous observation of the animals' intake behavior. The remaining feed was delivered by an automatic feeder operating continuously until 18:00 h. Feed losses, assessed by daily inspection, were considered to be negligible. This feeding regime was designed in order to achieve verge-of-excretion of faecal pellets at around 09:00 h, timing which aided the collection of faeces for rheological measurements.

The fish were of conventional, unspecified microbiological status and housed in an experimental facility in 18 similar blue rounded-corner fiberglass tanks (diameter: 640 mm, height: 650 mm, water capacity approximately 0.22 m^3) at the Fisheries Research Station of Baden–Württemberg in Langenargen. Final rearing densities were between 37 and 46 kg m^{-3}. Before the start of the experiment, randomly selected fish were examined for bacteriological, virological, and parasitological pathologies by a federal veterinary institute (Staatliches Tierärzliches Untersuchungsamt Aulendorf).

The flow-through operated system was supplied with water from a groundwater well, free of fish pathogens and degassed by aeration and oxygenated before entering the tanks. The flow rate for each tank was adjusted to 3 L min^{-1}, and the photoperiod was fixed at 12L : 12D (lights on between 07:00 h and 19:00 h with a sigmoid transition through twilight over 10 min). Lumilux® daylight lamps provided 200 lx at the water surface. Oxygen concentration and temperature were maintained at 12 mg O_2 L^{-1} and 11.5°C, respectively, and monitored continuously at the outlet of three tanks. German standard methods were used to determine further water parameters as follows (range; mean ± standard deviation): pH ≈ 7.75; NH_4–N 193 ± 7.7 [μg L^{-1}] dissolved CO_2 ≈ 7.0 ± 0.0 [mg L^{-1}].

2.2. Fish Performance and Digestibility. At the beginning of the experiment, each fish was anaesthetized with clove oil concentration: 0.1 mL L^{-1}, exposure time: ca. 60 s), weighed, measured, and visually inspected for external disorders. At the end of the experiment, the fish were anesthetized by a sharp blow to the head after a clove oil bath (concentration: 0.1 mL L^{-1}, exposure time: ca. 60 s), weighed, and sacrified by means of a gill cut.

The feed conversion ratio (FCR), specific growth rate (SGR), and thermal growth coefficient (TGC) were calculated according to the following equations:

$$\mathrm{FCR} = \frac{\text{Feed [kg]}}{\text{Weight gain [kg]}}, \tag{1}$$

$$\mathrm{SGR}\left[\%\mathrm{d}^{-1}\right] = \frac{\ln_{(\text{mean final weight})} - \ln_{(\text{mean initial weight})}}{t_{(\text{mean final date})} - t_{(\text{mean initial date})}} \times 100, \tag{2}$$

Table 1: Crude and chemical composition of experimental diets.

Diet	Unit	1	2	3	4	5	6
		FB		HEM		PB	
Fish meal[a]	g kg^{-1}	330	330	199	199	—	—
HP soy[b]	g kg^{-1}	190	190	190	190	190	190
Corn gluten[c]	g kg^{-1}	100	100	100	100	100	100
Hemoglobin meal[d]	g kg^{-1}	—	—	100	100	—	—
Pea concentrate[e]	g kg^{-1}	34	34	15	15	275	275
Wheat gluten[f]	g kg^{-1}	—	—	—	—	150	150
Wheat[g]	g kg^{-1}	166	166	189	189	42	42
Fish oil[h]	g kg^{-1}	105	105	114	114	—	—
Rapeseed oil[i]	g kg^{-1}	100	100	100	100	213	213
Lecithin[j]	g kg^{-1}	—	—	—	—	10	10
Methionin[k]	g kg^{-1}	—	—	2.2	2.2	3.8	3.8
Vitamin premix[l]	g kg^{-1}	7.1	7.1	7.1	7.1	7.1	7.1
L-Lysin HCL[m]	g kg^{-1}	—	—	—	—	4.2	4.2
MCP (monocalcium phosphate)[n]	g kg^{-1}	—	—	10.6	10.6	20.5	20.5
Betafin[o]	g kg^{-1}	0.42	0.42	0.42	0.42	0.42	0.42
Attractant[p]	g kg^{-1}	5	5	5	5	5	5
Yttrium premix[q]	g kg^{-1}	0.2	0.2	0.2	0.2	0.2	0.2
Guar gum[n] (HV109)[r]	g kg^{-1}	—	3	—	3	—	3
Moisture	g kg^{-1}	-3.73	-3.73	-3.3	-3.3	-2.1	-2.1
Sum	**g kg^{-1}**	**1000.0**	**1003.0**	**1000.0**	**1003.0**	**1000.0**	**1003.0**
Chemical composition (dry matter)							
Protein	g kg^{-1}	436	437	451	450	447	452
Fat	g kg^{-1}	216	209	201	202	208	207
Starch	g kg^{-1}	158	169	134	127	130	136
Phosphorous	%	0.92	0.96	1.01	0.95	0.99	1.02
Maximum extrusion temperature (at feed matrix)	°C	84.0		84.0		88.0	
Gross energy	kJ g^{-1} DM	24.7	24.1	24.4	25.1	24.9	24.7
Digestible energy[s]	kJ g^{-1} DM	18.4	19.1	19.5	19.4	19.3	19.2

[a]Unknown. [b]Oelmühlen Hamburg Aktiengesellschaft, Germany; soybean meal (dehulled) (48% protein). [c]Corn gluten meal (60% protein), Tate&Lyle, USA. [d]Unknown. [e]Unknown. [f]Amytex 100 (80% protein, 5% fat, max 1.5% ash), Amylum, Belgium. [g]Bread-making quality (*Triticum aestivum*, origin: Sweden). [h]SR fish oil, SR-mjöl, Iceland (mainly blue whiting). [i]Unknown. [j]Unknown. [k]Degussa, Germany. [l]Farmix, Putten, Netherlands. [m]Ajinomoto Eurolysine, France. [n]Kermira GrowHow Oyi, Finland. [o]Betafin S1, Danisco, Denmark; 96% betaine anhydrate. [p]Unknown. [q]Farmix, Putten, Netherlands. [r]SEAH International, France. [s]DE, calculated according to chemical analyses and digestibility measurements: lipids (39.5 kJ g^{-1} DM), proteins (23.6 kJ g^{-1} DM), and carbohydrates (17.2 kJ g^{-1} DM) [17].

$$\text{TGC} = \left(\frac{\sqrt[3]{\text{Final weight [kg]}} - \sqrt[3]{\text{Initial weight [kg]}}}{\text{days} * \text{average daily temperature [°C]}} \right) * 1000. \tag{3}$$

2.3. Sampling of Faeces and Digestibility. Faecal samples were collected from several tanks per day from 08:00 to 11:00 h after fish had been killed as described above. Mucus sheathed faecal pellets only were removed by intestinal dissection from hind gut. Sampling of faeces for digestibility measurements and final weighing was done after 100 days. Faeces were frozen immediately after collection (-20°C) then lyophilized and homogenized in order to determine dry matter (DM) and yttrium oxide content. Dry matter content was determined as the ratio of dry to wet weight after lyophilization (±0.1 mg). Yttrium oxide levels were determined using inductively coupled plasma mass spectrometry (ICP-MS), at the federal chemical analysis service of Baden-Württemberg (Chemisches und Veterinäruntersuchungsamt Sigmaringen, Germany). Samples were prepared as described in [18]. The apparent digestibility coefficients (ADC) for protein, fat, starch, and phosphorus were calculated as follows:

$$\text{ADC (\%)} = \left(100 - \left(100 * \frac{Y_2O_{2(\text{diet})}}{Y_2O_{2(\text{faeces})}} * \frac{\%\text{nutrient}_{(\text{faeces})}}{\%\text{nutrient}_{(\text{diet})}} \right) \right). \tag{4}$$

Sampling for PSD and rheological measurement was done at the end of the experiment, over a period of four days, due to the time requirement for particle size analysis and rheology. Dissected faeces was kept in aluminum dishes at 4°C to prevent

Table 2: Nutrient digestibility and growth parameters (mean ± SE) of the different diets.

Origin	Guar gum	Protein ADC ($n = 18$)	Lipid ADC ($n = 18$)	Starch ADC ($n = 18$)	Phosphorus ADC ($n = 18$)	Weight gain ($n = 18$)	FCR ($n = 18$)	SGR ($n = 18$)	TGC ($n = 18$)
FB	—	91.2% ± 0.3%^{b}	93.9% ± 1.8%	83.0% ± 0.1%^{ab}	46.8% ± 2.4%^{b}	200 ± 4.0	0.92 ± 0.02	1.32 ± 0.03	2.57 ± 0.04
	0.3%	89.9% ± 0.1%^{c}	89.5% ± 0.8%	79.4% ± 1.2%^{b}	51.5% ± 0.4%^{b}	182 ± 5.0	0.99 ± 0.02	1.27 ± 0.02	2.42 ± 0.04
HEM	—	91.2% ± 0.5%^{bc}	89.3% ± 2.7%	86.1% ± 0.5%^{a}	59.2% ± 1.2%^{a}	169 ± 6.3	1.04 ± 0.03	1.23 ± 0.03	2.32 ± 0.05
	0.3%	90.2% ± 0.6%^{bc}	93.1% ± 1.6%	86.0% ± 0.6%^{a}	60.6% ± 1.4%^{a}	177 ± 8.1	1.04 ± 0.03	1.25 ± 0.01	2.37 ± 0.04
PB	—	93.2% ± 0.3%^{a}	95.4% ± 1.1%	70.9% ± 1.3%^{c}	66.0% ± 0.9%^{a}	189 ± 1.9	0.97 ± 0.02	1.29 ± 0.01	2.49 ± 0.02
	0.3%	94.5% ± 0.3%^{a}	92.3% ± 1.0%	72.3% ± 0.5%^{c}	67.8% ± 2.7%^{a}	192 ± 0.7	0.96 ± 0.02	1.29 ± 0.01	2.50 ± 0.01
Model effects									
Origin		***	ns	***	***	ns	ns	ns	ns
Binder		ns	ns	ns	ns	ns	ns	ns	ns
Interaction		**	ns	*	ns	ns	ns	ns	ns

Table 3: Crude composition (DM) and faecal dry matter content of faeces.

Origin	Guar gum	Protein ($n = 18$)	Lipid ($n = 18$)	Starch ($n = 18$)	Phosphorus ($n = 18$)	Dry matter ($n = 18$)
FB	—	19.0% ± 0.4%^{b}	5.8% ± 1.8%^{a}	11.0% ± 0.1%^{ab}	2.4% ± 0.1%^{b}	9.5% ± 0.01^{bc}
	0.3%	20.7% ± 0.6%^{c}	9.6% ± 0.7%^{a}	11.8% ± 1.2%^{b}	2.2% ± 0.1%^{b}	10.1% ± 0.2^{ab}
HEM	—	19.8% ± 0.8%^{bc}	12.0% ± 3.0%^{a}	11.5% ± 0.5%^{a}	2.0% ± 0.1%^{a}	9.2% ± 0.1^{c}
	0.3%	20.1% ± 1.2%^{bc}	6.7% ± 1.5%^{a}	11.2% ± 0.6%^{a}	1.7% ± 0.1%^{a}	10.3% ± 0.1^{a}
PB	—	13.8% ± 0.6%^{a}	4.3% ± 1.1%^{a}	17.0% ± 1.3%^{c}	1.6% ± 0.1%^{a}	8.4% ± 0.3^{d}
	0.3%	11.0% ± 0.7%^{a}	7.0% ± 0.9%^{a}	16.7% ± 0.5%^{c}	1.4% ± 0.1%^{a}	9.9% ± 0.2^{abc}
Model effects						
Origin		***	ns	***	***	***
Binder		ns	ns	ns	ns	***
Interaction		**	ns	**	ns	*

microbial degradation and dehydration. PSD analysis and rheology were performed within 8 hours of dissection.

2.4. Particle Size Distribution (PSD). For the particle size determination, samples were processed immediately after collecting. 3 g (wet weight) of faeces was placed in a 2 L distilled water tank and exposed to defined and consistent shear forces resembling the turbulence in a fish farm [19], created by a constant air stream from below with air pressure maintained at 0.05 MPa for 480 seconds. PSD was measured in duplicate for each tank using a noninvasive laser particle sizer (GALAI:CIS-1) equipped with a flow controller (GALAI:LFC-100) and a flow-through cell (GALAI:GM-7) according to Brinker et al. [19].

2.5. Rheological Measurements. Excised faecal casts were cooled and kept under humid conditions to avoid dehydration until measurement. For each measurement, 3 g (wet weight) of faeces were carefully transferred to the rheometer (Paar Physica-Physica UDS 200). Measurements were done in duplicate or triplicate when sufficient sample was available, according to Brinker [20]. The plate-to-plate set-up consisted of a riffled plate with a 50 mm diameter (MP 313, Paar Physica), with a measuring gap width of 1 mm. The shear stress factor was 2.037, and shear force factor was 2.617. For the time sweep, a deformation with an amplitude of $\gamma = 30\%$ and a frequency of 1 Hz was applied. Mean values of dynamic viscosity and storage modulus were merged in order to obtain the dimensionless parameter "stability" reflecting viscous and elastic properties of the measured faeces.

2.6. Data Analysis and Statistics. Data were checked for homoscedasticity using Leven's test [21] and normality by visual inspection of the distribution followed by a goodness of fit test [22]. For the differences in continuous response variables, a nested ANOVA was applied with the variable *tank* as a random factor. All pair post hoc comparisons were made using Tukey's HSD test [23].

All descriptive statistics and linear regression analyses were calculated according to [24] using JMP Pro (SAS Institute Inc.), Version 14.

3. Results

3.1. Fish Performance. Feeding behavior and feed intake were similar for all treatment groups. Overall, rainbow trout of all treatment groups showed good growth within economically viable ranges for commercial diets as summarized in Table 2. No differences in feed conversion, TGC, SGR, or weight gain were observed. Effects of protein origin on nutrient digestibility were particularly pronounced for phosphorus, with distinctly lower values for the standard fish meal group, while the starch digestibility was relatively low in the all-plant group compared to the other diets. No statistical effects of guar gum on growth were observed. Survival did not differ statistically between groups and were high in all cases, at 100% for the standard fish meal and the hemoglobin diets and 99% for the vegetarian group.

3.2. Composition and Consistency of Faeces. The dry matter content of faeces was strongly influenced by dietary treatment, with significantly lower values for the pure plant diets. Furthermore, binder presence indicated a clear increase of dry faecal matter in all observed treatment groups (Table 3).

Starch content was significantly higher in faeces resulting from all-plant diets, but protein content was much lower. No differences in faecal lipid levels were observed between treatments, but phosphorus levels were significantly elevated in the fish meal group.

Apart from differences in color, visual inspection of faeces showed clear effects of dietary source and binder inclusion on consistency (Figure 1). Diets including hemoglobin meal appeared to lead to the most resistant faeces, while pellets resulting from the all-plant treatment appeared bulkier and less instable. Stabilizing effects of guar gum inclusion were clearly visible for all dietary treatments.

Mean stability values resulting from rheological measurements were consistent with the visual impression of faecal consistency, with the highest values recorded for faeces resulting from the binder-stabilized standard and the hemoglobin treatment (Figure 2), followed by the respective treatments without guar gum. The mean stability values of faeces resulting from the all-plant diet without guar gum addition were more than three times lower than those of the most stable standard treatment; however, the addition of guar gum

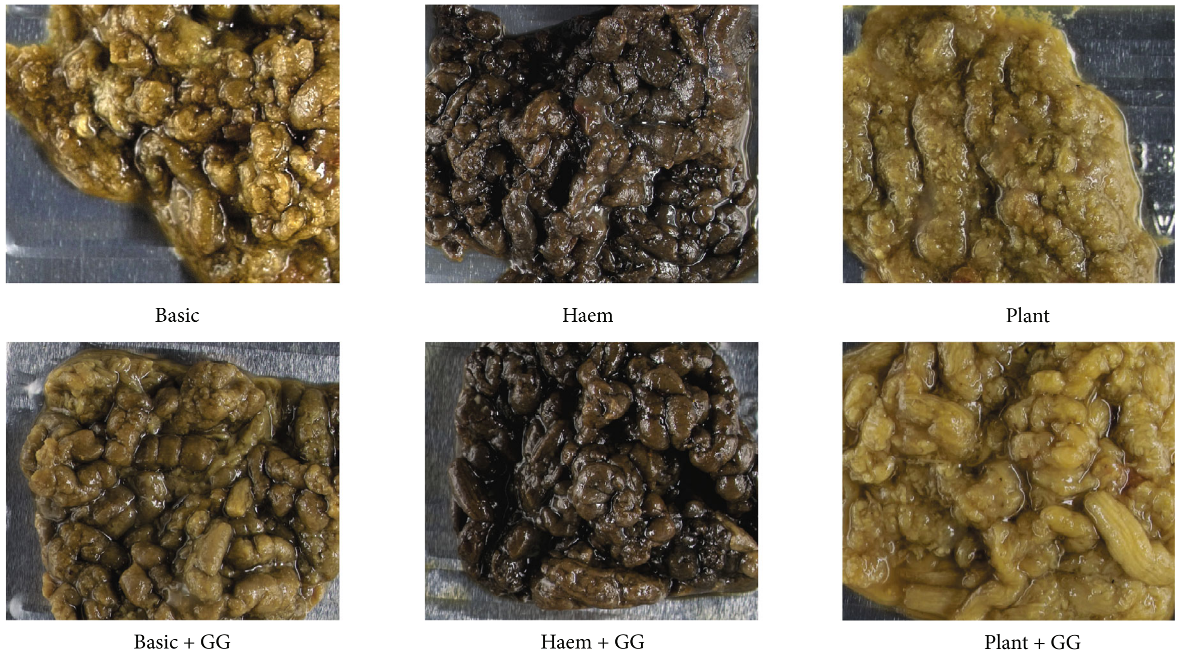

Figure 1: Faeces from rainbow trout fed different dietary treatments each without (upper row) and with addition of 0.3% of guar gum (bottom row).

brought values close to those seen in the hemoglobin diet without guar gum.

3.3. Particle Size Distribution. Cumulative PSDs for the standard and blood meal diets broadly reflected the observed stability values (Figure 3), with distribution curves indicating slight differences between the two treatments and a clear shift to larger particles resulting from guar gum addition. The PSD curves generated by the unstable faeces resulting from the plant-based diets both with and without guar gum showed distinctly steeper shapes across the whole size spectrum and point to a greater proportion of small particles.

The effect of guar gum inclusion is not uniform across the range of particle sizes for the plant-based diet, only coming into significant effect for size classes larger than 200 μm and almost nonexistent for particle sizes between 40 and 100 μm, the most relevant range for treatment by drum filters. The proportion of particles of 612 μm approaches that results from the other treatments without binder inclusion.

This phenomenon is confirmed by data shown in Figure 4, illustrating the relative effects of guar gum inclusion on particle size classes most relevant to solid treatment. In the controlled agitation experiments, the inclusion of dietary binder led to a greater proportion of particles of 200 μm size class in all three protein treatments. The standard fishmeal treatment displayed the greatest potential for improvement in shear resistance at more than 40%, while the scope for improvement was lowest in the plant-based treatment. Shares of smaller particle size classes could also be considerably reduced by guar gum in the standard and the hemoglobin treatment, but the effect was lost in the all-plant diet.

3.4. Effects on Solid Treatment Efficiency. Figure 5 shows the cumulative particle size distributions of the standard and the plant-based protein treatments with dietary guar gum and the theoretical single-pass removal efficiency using the 100 μm gauze commonly applied in drum filters. While only 12% of faecal particles generated by the standard diet with guar gum theoretically pass a 100 μm gauze, the share is around three times higher for the plant-based diet with guar gum, at 31%.

Theoretical particle removal efficiencies calculated for drum filter screening and sedimentation are presented in Table 4 and reveal far greater removal treatability for the standard and the hemoglobin treatments than the vegetarian diet. Guar gum had no effect on removal efficiency by filtration for the pure plant diet while slight improvements of around 4-6% were suggested for both other treatments. Differences in theoretical sedimentation efficiency were more pronounced, with best results associated with the hemoglobin diet and very low efficiencies for the plant-based treatment. Accelerating settling as a result of guar gum improved theoretical efficiency for all treatments, but the values for the all-vegetarian approach remained relatively low.

4. Discussion

4.1. Fish Performance. Fish performance data indicated that neither dietary treatment nor guar gum inclusion affected any of the investigated parameters. However, the analyses of nutrient digestibility revealed some differences, with both pure plant treatments exhibiting distinctly lower digestibility for starch and much higher digestibility for protein. Even when purified to a high extent, plant protein concentrates contain a proportion of complex indigestible carbohydrates including resistant starches and insoluble fibers, the presence of which may account for the reduced starch digestibility seen in the plant-based diets here [26, 27].

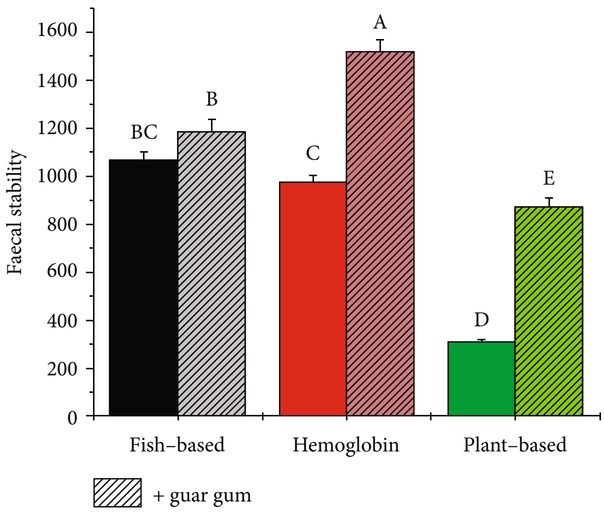

Figure 2: Dimensionless stability (merged mean values of measured dynamic viscosity and elasticity ± SE; $n = 337$) resulting from time sweep measurements of faeces from rainbow trout of respective protein treatment with and without guar gum. Different letters above bars indicate statistical difference (Tukey-Kramer HSD).

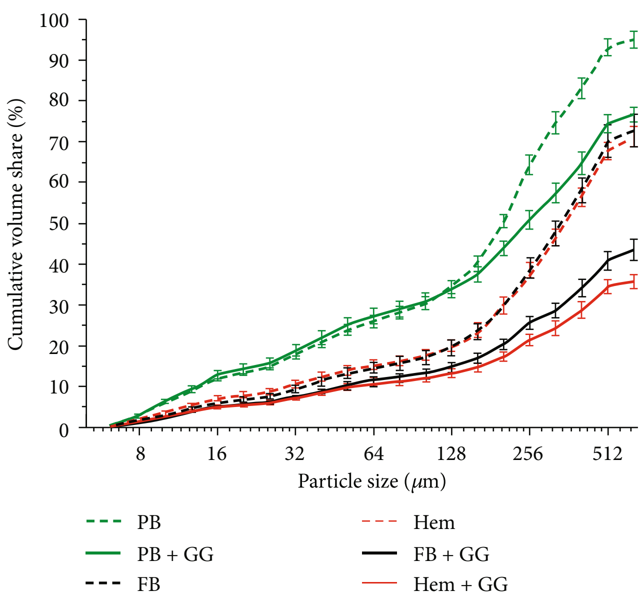

Figure 3: Cumulative volume percentage of faecal particle size distribution after controlled disturbance experiments of each tested diet.

Otherwise, provided they are processed adequately and antinutritive factors are removed, plant proteins are known to be highly digestible and potentially more so than fishmeal [28]. The pea protein concentrate and wheat gluten used in the current study are both highly digestible and likely responsible for the high protein usability of the pure-plant treatment. Phosphorus digestibility was higher in the plant-based and hemoglobin diets compared to the basic fish meal mix. This was expected, as the natural deficit in plant ingredients and hemoglobin meal [14] was corrected using highly available phosphorous supplements that precisely met the requirements of the fish while the surplus available in fish meal diets would have been excreted and not digested [29].

Despite these observed differences, nutrient digestibility did not affect overall growth performance of fish, which all fell in usual range for commercial production. While a nonsignificant tendency to decreased growth and feed conversion was observed for the hemoglobin treatment, the vegetarian diets performed equally well to the fish meal treatment. In terms of fish performance and by excluding other factors such as flesh quality, fish health, water quality, or costs, the present study suggests that a well-balanced diet based only on vegetarian ingredients is a competitive alternative to a standard fish meal diet. However, it has to be mentioned that the production period was limited, and feeding of fish was restrictive.

4.2. Faecal Composition and Consistency. Of the dietary treatments tested in the current study, the stability data clearly indicate a strong tendency for faecal disintegration when the feed comprised only plant ingredients. Similar results were found for diets based on plant proteins that still included fish/marine-based oil fractions [10, 30].

A functional explanation for the instability of faeces from plant-based ingredients is lacking due to the scarcity of data and the complexity of feed composition. However, the effect may be attributable to single factors or properties. Of the diets used in the present experiment, only the plant-based formulations contained pea protein concentrate or wheat gluten. While wheat gluten is a highly digestible feed ingredient with a very low fiber content [14], protein concentrates (PC) including those derived from legumes like pea or soy as well as grains contain a significant quantity of resistant starches (RS) and insoluble fibers (IF) including cellulose or hemicellulose which originates from cell walls. These indigestible feed components are also reflected in a higher level of starch and lower shares of protein in faeces resulting from the pure-plant treatments (Table 3).

Insoluble fibers and resistant starch are known to have a relatively low impact on viscosity of digesta compared to the soluble fiber fraction, but they can affect faecal consistency. For monogastric animals, IFs and RS are largely indigestible and in humans, rats, and fishes for example, they lead to bulking of faecal volume, increased moisture content, and reduced density [31]. In this context, laxation and loose stools/faeces are widely observed side effects. This is consistent with faecal instability observed as a result of the pure plant diets in the current study. However, the underlying mechanism have to be reassessed. For decades, the increase in faecal water content was attributed in part to the high water holding capacity of insoluble fibers [32–34]. More recently, however, McRorie & McKeown [35] have suggested that water holding capacity of IFs are generally too low to explain bulking and loss of faecal stability [36] and that the increased water content in faeces may be due to irritation of the large bowel mucosa by IF, triggering secretion of water and mucus as a defensive mechanism. If so, the bulking and water content may be more attributable to particle coarseness than their water binding properties. This theory is supported by studies in which similar bulking and laxative effects were achieved by plastic particles mimicking effective IF in size and shape without any

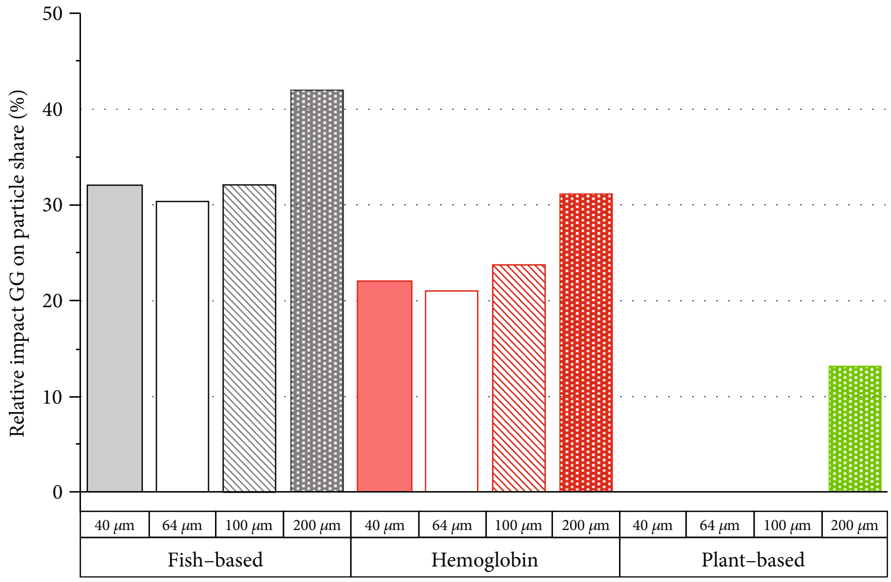

Figure 4: Relative change of 0.3% guar gum addition on cumulative particle shares of selected size classes for the tested dietary treatments.

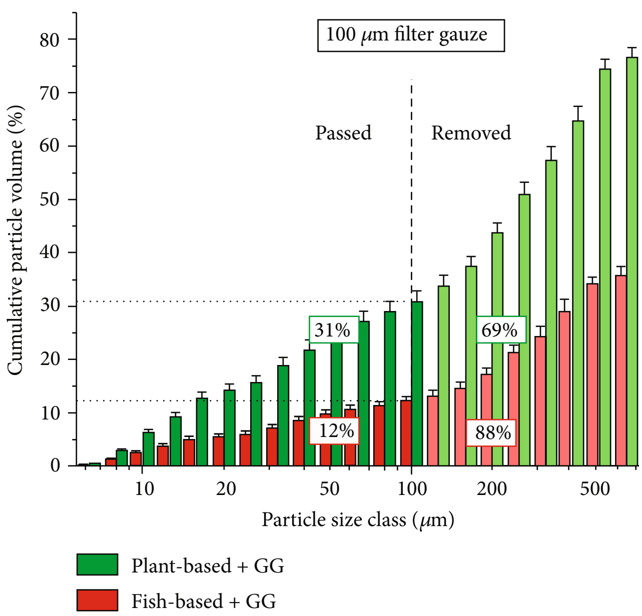

Figure 5: Particle size distribution of two selected diets and calculated drum filter removal efficiencies when a 100 μm gauze is applied.

inherent water binding capacity at all [37, 38]. The resulting faeces are often described as bulky, soft, and unstable with a low density, but reproducible mechanical measurements of faecal consistency, i.e., by rheological means, are lacking. In this study, we used the well-established shear rheology and merged the parameters viscosity and elasticity to arrive at a dimensionless parameter "stability," to describe the resistance of faecal material to shear forces in aquaculture [20]. The reduced stability of faeces resulting from plant-based feeds observed in the present and previous studies [10] support the proposed mechanism for fish diets containing high proportions of plant protein concentrates. Furthermore, there is increasing evidence that carbohydrates not contributing to viscosity of digesta or faeces can increase faecal water content in fish. Refstie, Svihus, Shearer, & Storebakken [39] tested salmon diets formulated with soy bean products processed differently with varying levels of nonstarch polysaccharides (NSP), and while viscosity values of digesta did not change, faecal dry matter content decreased markedly with increasing NSPs.

The reduced transit time associated with IF content can amplify increases in faecal water content. Studies show that indigestible fibers (shape and size dependent) decrease transit time, and thereby inhibit water reabsorption in the hind gut [40]. The opposite applies for fibers or gums, which increase the viscosity of digesta: slower transit through the digestive tract reduces faecal water content by allowing more time for reabsorption may thus explain the higher faecal dry matter content of guar gum treatments in this study, despite its intrinsic hydrating properties. A further comprehensive meta-analysis (data in preparation) reveals a general increase in faecal dry matter content from fish consuming diets with low levels of highly viscous, fast-hydrating guar gum compared to diets without binders.

The loss of stability could also be exacerbated by the low protein levels in the pure plant faeces. Many proteins have relatively high water-binding capacities, based for example, on structural characteristics such as number of polar groups or globular conformation [41]. The replacement of a rheologically significant protein fraction by bulking material with less pronounces or even nonexistent effects on consistency is bound to impact on faecal stability.

The ideal feed ingredient contributes to the nutritive value of a feed and imparts beneficial functional properties. Hemoglobin meal is one such candidate, used as a protein source and/or binder in animal feeds [42, 43].

A look at stability data yielded by the current study shows a positive effect of hemoglobin meal in combination with guar gum, while the treatment without binder performs

Table 4: Calculated single-pass removal efficiencies of drum filter and sedimentation basin with two overflow rates (ORF in cm s^{-1}) within ranges from literature [25].

Origin	GG	Drum filter 60 μm	Drum filter 100 μm	Sedimentation OFR 0.02	Sedimentation OFR 0.15
FB	—	85%	82%	64%	61%
	0.3%	89%	88%	79%	78%
HEM	—	85%	83%	57%	70%
	0.3%	89%	87%	86%	95%
PB	—	74%	70%	40%	20%
	0.3%	73%	69%	54%	36%

just as well as the fish-based diet. The exact mechanism of this effect remains unclear but an obvious hypothesis may be a synergistic interaction of guar gum and the globular hemoglobin proteins similar to that known to occur in other globular proteins contained in soy products [44].

4.3. PSD. Rheological measurements provide a reliable method of detecting feed-induced differences in faecal stability in the fish aquaculture setting [13], and several previous studies indicate a strong correlation of rheological data with PSD in both experimental setups and commercial systems [19, 45]. However, as observed by Brinker & Friedrich [10] and the current study, this functional relationship seems to break down for feed mixtures with high plant protein content. While binder addition can partly or fully compensate the loss of faecal stability, the change is not fully expressed in PSDs. Despite a more than threefold increase in stability observes when guar gum was added to the all plant treatment in this study, a shift in particle distribution towards larger particles was only apparent for particle sizes above 120 μm well outside the range needed to make a practical difference for solid treatment [46]. This undesirable break-down of faecal matter poses a significant problem in aquaculture, and clarification of the mechanisms behind it is much needed in order to mitigate and further optimize feed composition especially given the increasing pressure to reduce use of fish meal.

4.4. Consequences for Treatment Efficiency and Other Parameters. Particle size distribution and density are decisive factors for efficiency of the mechanical treatment systems applied in aquaculture. In the current study, theoretical values for the efficiencies of drum filtration calculated using size distribution data derived from the controlled agitation experiment and density data measured in previous studies [25] show a very clear effect of feed composition on solid removal potential. The effects of faecal fragmentation go far beyond particles, impacting a variety of other relevant water parameters. When CO_2 levels exceed recommended values in RAS for salmonids, there are negative impacts on fish performance and health. While the main share of CO_2 in such systems is excreted from the gills of fish, for every kilogram of feed consumed, fish defecate around 250 g of solids, resulting in about 350 g of microorganism-mediated CO_2 production [12, 47]. Based on the PSD data and filter efficiency estimates presented here, the solids escaping the filter (100 micron) from the standard diet with guar gum would lead to around 40 g of CO_2 per kilo of feed consumed by the fish while the all-plant diet would generate more than 100 g of CO_2 per kilo. Thus, a high solid load can indirectly limit fish production in RAS.

5. Conclusion

The trials documented here show the potential of feed-induced optimization of faecal consistency. Up to now, little is known about how different factors at play in a complex matrix of fish feeds interact to affect faecal consistency. The systematic identification of stabilizing and destabilizing elements in different feed ingredients would be a major step towards a better understanding of the key mechanisms influencing the structure and stability of faeces.

Data Availability

The (fish sizes, composition of faeces, faecal stability, and particle size distribution) data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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