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Guar gum in rainbow trout (*Oncorhynchus mykiss*) feed: The influence of quality and dose on stabilisation of faecal solids

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Abstract

In a dose–response study to identify optimal binder inclusion parameters, a control diet and five diets incorporating binder (0.2, 0.3 and 0.4% of a mid-viscosity guar gum (MV) and 0.2%, and 0.3% of a high-viscosity guar gum (HV)) were fed to triplicate groups of rainbow trout at a ration of 1.2% BW d^{-1}. Feed conversion, growth, and with one exception, apparent digestibility of macronutrients were not significantly effected by the addition of binder. All binder containing diets improved stability of fish faeces significantly with the most effective (HV 0.3%) leading to increases in viscosity and elastic modulus of up to 266 and 209%, respectively. The high-viscosity guar gum (HV) performed significantly better than the mid-viscosity (MV), and improvements were positively dose- and quality dependent, except in the case of MV 0.4%, which yielded a slightly less marked improvement than MV 0.3%. The possibility of inactivation by microbial fermentation is discussed. Furthermore, compared to the control, suspended particles from binder-stabilized faeces remained significantly larger and therefore removable by mechanical means after exposure to defined hydrodynamic stress. According to the stability data recorded, the best result was achieved by supplementing feed with 0.3% high-viscosity guar gum. This resulted in a reduction in post-microscreening residual load of about 52% for a 100 μm gauze. The improvement correlated strongly ($r^2=0.95$) with measured faecal stability data, suggesting that final particle size in self-cleaning trout farms is in effect a measure of mechanical stability of faeces in the face of given turbulences.

It is suggested that the economic and ecological advances deemed essential in fish farm process management may be achieved through systematic manipulation of the physicochemical properties of faeces by certain NSPs.

Keywords: Effluent treatment; Binder; Guar gum; Rheology; Faeces; Dose; Shear resistance

1. Introduction

The process of fish production generates waste substances that are potentially harmful to both the interests of livestock husbandry and the environment (Baird et al., 1996; Bergheim and Åsgård, 1996). In fish farm systems, suspended particles comprising mainly fish faeces are a significant part of the total pollutant load (Summerfelt, 1998). A proportion of those solids can be removed mechanically by microsieves and sedimentation devices.

However, such cleaning methods are only effective if a significant fraction of the total load consists of particulate matter large enough to be retained by the relevant filter mesh (Cripps and Bergheim, 2000; Bergheim and Brinker, 2003). Thus one promising avenue of research in aquacultural effluent management involves the addition of dietary binders to fish feeds, resulting in the production of large faecal particles with an enhanced potential to

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retain leachable components (USDA/CSREES, 1997; Brinker et al., 2005a). These binders (indigestible non-starch vegetable or microbial polysaccharides — NSPs) need only be added to the feed in small doses, as they concentrate along the intestine and only reach an effective concentration after water absorption and final compaction in the far distal intestine (Brinker, 2005). This property conveniently optimizes the economic viability of the technique, while minimizing the risk of unwanted side effects during the digestion processes.

Previous field trials carried out at a commercial land-based trout farm have proved that the addition of guar gum to a commercial trout diet significantly improved drum filtration efficiency for total suspended solids (TSS), total phosphorus (TP), and total Kjeldahl nitrogen (TKN) (Brinker et al., 2005b). However, since the quality of commercially available guar gum varies and its effects are strongly concentration-dependent, a response study is appropriate in order to test for the most effective combination of quality and dose.

Guar gum is a linear polysaccharide (galactomannan) derived from the endosperm of the Indian cluster bean (*Cyamopsis tetragonolobus*). Chemically, it is based on a backbone of $\beta(1\rightarrow4)$-linked D-mannose residues with single linked $\alpha(1\rightarrow6)$-D-galactose. The viscosity of the compound is a property of the hydroxyl groups (Fox, 1992) and of branching of the structure. The gum shows differing degrees of polymerisation and is available in different grades in which cold viscosity (at 1% solution in demineralised water, 25 °C, 1 h) ranges from 0 (depolymerised guar gum) to 10.000 mPa s. The performance of the gum depends on several factors, including purity, origin, variety of constituent seeds, soil condition and various production parameters including pressure/shear which are not generally published by manufacturers (Hill et al., 1998).

In solution guar gum adopts a flexible coil-like structure but at concentrations exceeding the dimensionless overlap factor c* interpenetration of individual polymer coils occurs (Hill et al., 1998). This is the concentration desirable in faeces, having proved effective for stabilising of food mixtures which are comparable to chyme/faeces in many ways (Fox, 1992).

2. Materials and methods

2.1. Diets and husbandry

Six isonitrogenous and isoenergetic diets were formulated, all based on the same basic feed containing balanced levels of essential amino acids, fatty acids, vitamins and minerals exceeding the levels recommended by National Research Council (1993). One negative control lacked binder and the others contained binder of two different grades in different concentrations (Tables 1, 2).

The diets were produced using a Wenger preconditioner and a Wenger single screw extruder (X-85) with a 5 hole dieplate (each hole has a diameter of 4.5 mm, setpoints for the preconditioner: steam 3%, water 15%; for the extruder: water 16%; extruder barrel setpoint: 75 °C; maximum extrusion values at feed matrix: 80 °C, 16 MPa). This took place in the Nutreco Technology Centre. In order to maintain maximum homogeneity between treatments, the binders were added on top of the basal mix. Dilution effects were minimal as can be seen from chemical analyses of the finished feeds (Table 1). As a positive control, in one experiment, diet 2 was replaced by a commercial feed (Skretting/Royal Optima® 3P-TROUW Nutrition Deutschland GmbH, Burgheim, Germany described as: crude protein 44.0, crude fat 28.0, nitrogen-free extract 10.4, crude ash 9.0, crude fibre 0.6, phosphorus 1.0, digestible energy 21.3 MJ kg^{-1}). The commercial feed was treated with 1 g Yttrium oxide per kg, dispensed in 5 g rapeseed oil and mixed for 180 s in a chum.

For each of the six diets tested, three experimental trials were conducted with triplicate groups of rainbow trout (*Oncorhynchus mykiss*). Each trial measured the effects of the dietary binder treatments on a different set of parameters: trial 1 used groups of 28 fish per tank and ran for 14 days, during which the digestibility of the various feeds was assessed. In this trial, the positive control feed was used as described above; trial 2, on groups of 24 fish per tank, ran for 32 days, during which tests were performed to ascertain faecal stability; trial 3 involved groups of 30 fish per tank and ran for 37 days, long enough to yield a doubling of body weight and generate data on specific growth rate (SGR), feed conversion ratio (FCR) and particle size.

The fish were fed 6 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 and 08:40 h under continuous observation of the animals' intake behaviour. The remaining feed was then delivered by an automatic feeder, which operated continuously until 18:00 h. This feeding regime resulted in the production of faecal pellets on the verge of excretion at around 09:00 h.

Each of the five experimental treatments (diets 2–6, Table 1) contained one of two different grades (Table 2) of commercial guar gum (mid-viscosity MV and high-viscosity HV) in different concentrations. The

Table 1
Composition of the experimental diets (BD=basic diet, MV=mid-viscosity guar gum, HV=high-viscosity guar gum)

	Unit	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
		BD	MV 0.2%	MV 0.3%	MV 0.4%	HV 0.2%	HV 0.3%
Fish meal [a]	g kg^{-1}	329.7	329.7	329.7	329.7	329.7	329.7
Soybean meal [b]	g kg^{-1}	200.0	200.0	200.0	200.0	200.0	200.0
Wheat [c]	g kg^{-1}	190.0	190.0	190.0	190.0	190.0	190.0
Corn gluten meal [d]	g kg^{-1}	100.0	100.0	100.0	100.0	100.0	100.0
Fish oil [e]	g kg^{-1}	159.0	159.0	159.0	159.0	159.0	159.0
Lupines [f]	g kg^{-1}	35.6	35.6	35.6	35.6	35.6	35.6
Wheat gluten [g]	g kg^{-1}	13.6	13.6	13.6	13.6	13.6	13.6
Mineral premix [h]	g kg^{-1}	1.1	1.1	1.1	1.1	1.1	1.1
Vitamin premix [h]	g kg^{-1}	1.1	1.1	1.1	1.1	1.1	1.1
Lysine*HCl [i]	g kg^{-1}	3.0	3.0	3.0	3.0	3.0	3.0
Carophyll pink [j]	g kg^{-1}	0.6	0.6	0.6	0.6	0.6	0.6
Yttrium premix [k]	g kg^{-1}	1.0	1.0	1.0	1.0	1.0	1.0
Betafin [l]	g kg^{-1}	0.42	0.42	0.42	0.42	0.42	0.42
Lutavit C Aquastab 35% [m]	g kg^{-1}	0.14	0.14	0.14	0.14	0.14	0.14
Moisture change	g kg^{-1}	−35.3	−35.3	−35.3	−35.3	−35.3	−35.3
Sum	g kg^{-1}	1000	1000	1000	1000	1000	1000
On-top coating							
Guar gum MV	g kg^{-1}	–	2.0	3.0	4.0	–	–
Guar gum HV	g kg^{-1}	–	–	–	–	2.0	3.0
Chemical composition (dry matter)							
Crude protein	g kg^{-1}	431.3	418.8	443.8	450.0	400.0	443.8
Crude lipid	g kg^{-1}	180.9	200.1	197.5	188.0	187.5	189.5
Total phosphorus	g kg^{-1}	10.2	10.2	10.4	10.5	10.0	10.1

[a] SR fish meal, SR-mjöl hf, Iceland (mainly blue whiting *Micromesistius poutassou*, low temperature drying, 70% crude protein).
[b] Dehulled soybean meal, Oelmühlen Hamburg Aktiengesellschaft (48% crude protein).
[c] Bread-making quality, (*Triticum aestivum*, origin: Sweden).
[d] Corn gluten meal, Tate and Lyle PLC London (60% crude protein).
[e] SR fish oil, SR–mjöl hf, Iceland (mainly blue whiting *Micromesistius poutassou*).
[f] Lupinen kernel (dehulled), Australia (*Lupinus angustifolius*).
[g] Amytex 100, Amylum, Belgium.
[h] According to National Research Council (1993), Farmix, Putten, The Netherlands.
[i] Ajinomoto Eurolysine, France.
[j] Carophyll pink 10%, DSM, The Netherlands.
[k] Yttrium oxide was added as marker for digestibility measurements, Farmix, Putten, The Netherlands (≈ Y_2O_3–Y 0.07 g kg^{-1}).
[l] Betafin S1, Danisco, Denmark.
[m] BASF, Ludwigshafen, Germany.

concentrations were chosen based on expected final concentrations calculated according to the theoretical considerations outlined in (Brinker et al., 2005a).

Rainbow trout (all female, Hofer strain) were housed in an experimental plant in 18 blue rounded-corner fibreglass tanks (diameter: 640 mm, height: 650 mm, water depth approximately 0.22 m^3). Maximum rearing density was about 40.0 kg m^{-3}. The fish were of conventional, unspecified microbiological status. Water free of fish pathogens was supplied from a groundwater well.

The water flow for each tank was adjusted to 3 L min^{-1} and the photoperiod was fixed at 12 L:12 D (lights on between 07:00 and 19:00 h with sigmoid de/increasing twilight of 10 min) providing 200 lx at the water surface (Lumilux® daylight lamps). Oxygen concentration and temperature were maintained at 12 mg O_2 L^{-1} and 11 °C, respectively, and monitored continuously at the outlet of three tanks (nos. 3, 10, 15). Further water parameters were determined according to German standard methods (mean±SD): pH was 7.6±0.19; NH_4–N 225±89.2 [µg L^{-1}], i.e. NH_3–N<2.3 [µg L^{-1}]; dissolved CO_2 9.9±6.2 [mg L^{-1}]).

2.2. Specific growth rate, feed conversion, digestibility

Each fish was weighed (±1 g) at the beginning of the experiment and at the end, immediately after killing. The specific growth rate (SGR) of the fish, was calculated as

follows, using the mean weights recorded at the beginning and end of the trial:

$$\mathrm{SGR}\ [\%\ \mathrm{d}^{-1}] = \frac{\ln_{(\text{mean final weight})} - \ln_{(\text{mean initial weight})}}{t_{(\text{final date})} - t_{(\text{initial date})}} * 100. \quad (1)$$

The feed conversion ratio (FCR) was calculated as:

$$\mathrm{FCR} = \frac{\mathrm{Feed[kg]}}{\mathrm{Weight\ gain[kg]}}. \quad (2)$$

In order to obtain digestibility data, 28 trout per tank were anaesthetized with clove oil (concentration: 0.1 mL L^{-1}, exposure time: ca. 60 s) between 08:15 and 10:00 h after 14 days of feeding. Faeces stripped from the rectum were immediately frozen in liquid nitrogen, lyophilized, and homogenized. Dry matter content, protein, fat, phosphorus and yttrium oxide content were determined. The apparent digestibility of protein, fat, and phosphorus was calculated using the following equation (Edin, 1918; Austreng et al., 2000)

$$\text{Apparent digestibility coefficient (ADC[\%])} = 100 - \left(\left(100 * \frac{Y_2O_{2(\text{diet})}}{Y_2O_{2(\text{faeces})}} \right) * \frac{\%\ \text{nutrient}_{(\text{faeces})}}{\%\ \text{nutrient}_{(\text{diet})}} \right). \quad (3)$$

Values for each parameter were determined in duplicate.

Dry matter content was determined as the ratio of wet to dry weight after lyophillization (±0.1 mg). Crude protein in the feed was analyzed according to Commission Directive 93/28/EEC, Kjehldahl method (Anonymous, 1993). Crude protein and phosphorus in the faeces were analyzed according to Brinker et al. (2005b). Crude lipid was analyzed according to Folch et al. (1957). Yttrium was determined by the Chemisches und Veterinäruntersuchungsamt Sigmaringen, B. W.'s federal chemical analysis service. The samples were first digested in 2 mL nitric acid 65% (suprapur), 1 mL hydrogen peroxide 30% (suprapur), and 5 mL distilled water heated in a microwave oven (to 120 °C within 15 min, held for 5 min; to 150 °C within 10 min, held for 5 min; to 180 °C within 15 min, held for 25 min), then diluted with nitric acid 4 vol.%, and finally analyzed using Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).

2.3. Sampling of faeces

Due to the investment of time required to perform particle size and rheological measurements, these were performed in alternate weeks. The fish were sampled daily from three to five randomized tanks from 08:15 to 09:30 h. Individual fish were selected at random, anaesthetized with clove oil (dose: 0.1 mL L^{-1}; exposure time: 60 s), and killed by a sharp blow to the head. The lowermost faecal pellet was removed by intestinal dissection. Only mucus sheathed faecal pellets were used (Fig. 1). The faeces were placed in aluminium dishes, hermetically sealed with a plastic film to prevent dehydration, and cooled to 4 °C to slow down microbial degradation. Livers were removed for testing at the same time. All measurements were performed within 8 h of dissection.

The faeces and intestine were inspected for signs of exudative enteritis, hemorrhagic enteritis or irritation of intestinal mucosa. The excised livers were carefully sliced, and examined for macro-pathological alterations including necrosis, anaemia, hyperaemia/haemorrhage, discoloration of ochre, and textural disintegration. The degree and extent of each alteration was scored according to the Index of Liver Lesion (ILL) scale described by Bernet et al. (1999) with the modification of (Brinker and Hamers, in press).

2.4. Rheological measurements

Rheological measurements were carried out on merged samples with a minimum volume of about 3 cm^3 faeces. These were transferred to a Paar Physica–Physica UDS 200 rheometer (Anton Paar GmbH, 73760 Ostfildern, Germany). The measuring system applied was a MP 313 (plate: ∅ 50 mm, 0°) with a gap width of 1 mm. The plate was carved into a star-shape to improve precision

Table 2
Basic data for guar gum binders used in experimental treatments

Guar gum type	Product specification	Characteristics Cold viscosity in demineralised water: (measured with Brookfield RVT viscosimeter, n° 4 spindle, 20 RPM–25 °C–1% solution)	Costs	Inclusion level [range]
Mid-viscosity (MV)	HV 105 (SEAH International); Product Code : 3303	After 1 h: 4.500–5.000 mPa s	2.00 € kg^{-1}	0.2–0.4%
High-viscosity (HV)	HV 109 (SEAH International); Product Code : 3309	After 1 h: 9.500–10.000 mPa s	2.75 € kg^{-1}	0.2–0.3%

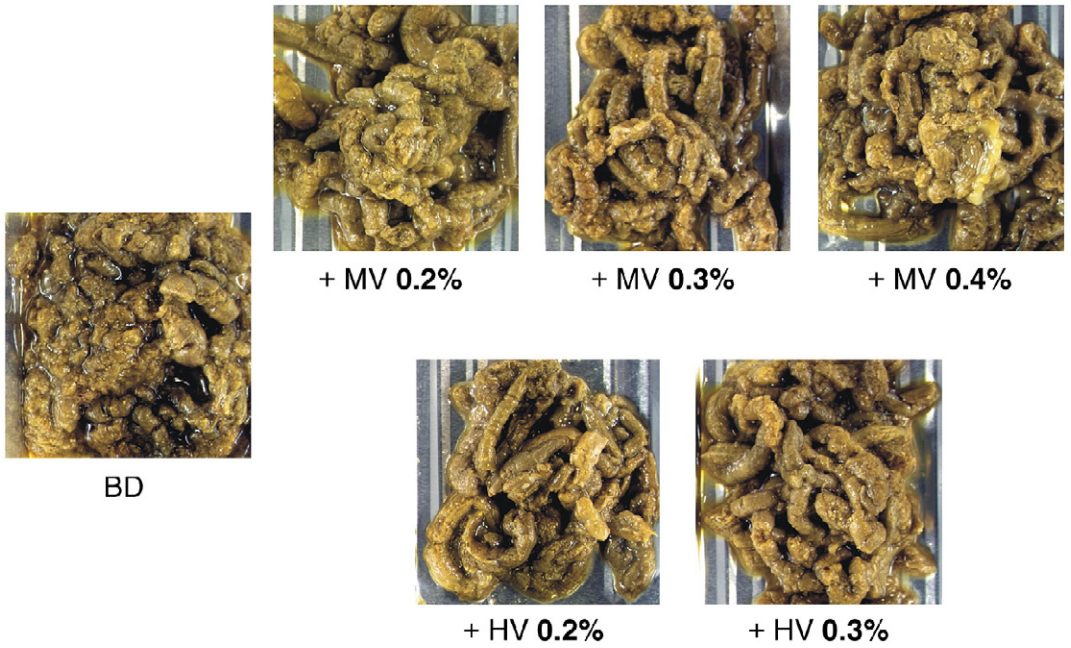

Fig. 1. Faeces from rainbow trout fed a basic diet (BD) and from trout fed the same diet but with the inclusion of various concentrations of two types of guar gum as binders (MV = mid-viscosity guar gum, HV = high-viscosity guar gum). Faeces were obtained by intestinal dissection. Note that the faeces containing binder are more structured and less pulpy than those resulting from the basic diet (BD).

and reduce the influence of water trapped within the measuring gap. The shear stress factor was 2.037 and shear rate factor was 2.617.

For the time sweep a deformation with an amplitude of $\gamma = 30\%$ at a frequency of 1 Hz was used. The duration of measurement was set at a logarithmic scale using 16 intervals (between 1 and 175 s log). For the studies in the frequency domain the samples were probed by sinusoidal excitation at frequencies of 50, 32.1, 20.6, 13.2, 8.47, 5.43, 3.49, 2.24, 1.43, 0.92, 0.59, 0.38, 0.24, 0.16 and 0.10 Hz. In each run the deformation amplitude was 10% and the duration of measurement was 30 s.

In the sample compartment the temperature was set to 4 °C and humidity was adjusted to 100% saturation. All measurements were deformation-controlled. Each measurement started with a time sweep of 16 single deformations, followed without delay by a frequency sweep.

2.5. Particle size distribution (PSD)

For particle size measurements, trout faeces were sampled as described above and measured according to Brinker et al. (2005c). For each measurement 3 g samples of faecal pellets were broken up in 2 L distilled water using turbulence generated by a constant air stream from below, using air pressure and agitation duration settings of 0.05 MPa and 480 s, respectively. Particle sizes were determined using a non-invasive 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. (2005c).

2.6. Data analysis

Differences in SGR, FCR, apparent digestibility of nutrients, dry matter content, and ILL were tested using Dunnett's method for comparing treatment to control (Dunnett, 1955). The Likelihood Ratio Chi-square test was used to test for differences in the frequency of occurrence of liver disorders. Rheological and particle size data were analysed using the following generalized model

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \kappa_k + \varepsilon_{ijkl} \quad (4)$$

where Y_{ijkl} is viscosity, elastic modulus, particle size; μ is the overall mean; α_I is fixed Diet treatment, β_j is fixed Concentration treatment; κ_k is the variable Tank nested within feed; $(\alpha\beta)_{ij}$ denote the interactions between the two treatments and ε_{ijk} is the random residual error. For the rheological data the model was extended by the time variable Measuring point as a random block factor. The first five points from the rheological data were excluded

Table 3
Effect of binder treatments on apparent digestibility coefficients (ADC) for protein, lipid, and phosphorus and on specific growth rate per day (SGR) and feed conversion ratio (kg feed per kg weight gain) (mean±SE)

Diet	Trial 1			Trial 3	
	Protein	Lipid	Phosphorus	SGR	FCR
	ADC (n=18)	ADC (n=18)	ADC (n=18)	(n=18)	(n=18)
Basic diet	89.2%±0.33%	83.7%±1.04%	48.3%±2.06%	1.10%±0.04%	0.987±0.040
Royal optima +MV 0.2%	90.6%±0.32%	84.3%±0.38%	48.6%±1.54%	1.08%±0.03%	1.010±0.035
+MV 0.3%	89.0%±0.32%	76.3%*±2.32%	54.2%±0.68%	1.08%±0.01%	1.014±0.005
+MV 0.4%	89.3%±1.34%	84.4%±1.37%	49.8%±7.32%	1.10%±0.01%	0.987±0.006
+HV 0.2%	88.5%±0.09%	79.1%±0.58%	53.5%±1.09%	1.08%±0.02%	1.001±0.017
+HV 0.3%	89.4%±0.09%	81.4%±0.56%	51.7%±0.77%	1.06%±0.02%	1.027±0.0245

Means within a column marked by an asterisk letter are different to basic diet (P<0.05).
In trial 1 the diet+MV 0.2% was substituted by commercial trout diet as a positive control (MV=mid-viscosity guar gum, HV=high-viscosity guar gum).

from the analysis in order to avoid anomalies resulting from the possible presence of undetected air bubbles within the samples — these would be eliminated by the first five agitations. Post-hoc comparisons were made by Tukey's HSD test (Hayter, 1984). The rheological data for the frequency sweep measurements were analysed using nested analysis of covariance (ANCOVA) with the variable Tank as a random factor (Sokal and Rohlf, 2003). The dependent variable Viscosity and the independent variable Frequency were $\log_{10}$ transformed to meet the assumptions of the model. Diet was included as a main factor, and the interaction of the variables Diet × $\log_{10}$ Frequency was used to check for treatment-dependent differences in slope.

The effect of binder quality (MV or HV) was tested using ANCOVA (dependent variable: merged values of viscosity and elastic modulus, independent variable binder inclusion level, binder quality (interaction term)).

The PSD data were arranged into size classes (d_i+1=1.26 d_i, d=upper diameter of class) according to Patterson et al. (1999). All data were converted into cumulative volume data assuming a sphere as basic shape. Inference statistics were performed for cumulative volume percentages at 100 μm and 600 μm, respectively. Individual pairwise comparisons of least squares means in the model were tested using sequentially Bonferroni-corrected Student's t-tests (Rice, 1989). Data were checked for homoscedasticity using Bartlett's test (Sachs, 1997). The correlations between stability and particle size were checked using reduced major axis regressions (Sokal and Rohlf, 2003).

The restricted residual maximum likelihood method was used to fit current models for random effects (Smyth and Verbyla, 1996).

All descriptive statistics and linear regression analyses were calculated according to Sachs (1997). All data analyses were done with JMP (SAS Institute Inc.), Version 5.1.2.

3. Results

3.1. Digestibility, specific growth rate, feed conversion

Three fish died during trial 3, one from a tank receiving the negative control diet and two from a tank receiving the experimental additive HV 0.3%. Otherwise, the fish developed normally and there were no visible signs of intestinal irritation or pathology that could have been attributed to the inclusion of dietary binder. The final weights were 205 g±38.9 g (trial 3), 274 g±38.9 g (trial 2), and 268 g±62.2 g (trial 1) (mean±SD). Liver analysis revealed disorders in 13% of all fish (specifically, anaemia in 3.3%, hyperaemia/haemorrhage in 0.27%, discoloration of ochre in 12.6%, but with no sign of necrosis or textural disintegration in any of the samples examined). None of the observed liver damage could be related to the binder treatments (P=0.376). The indices of liver lesion (ILL) among impaired livers were in the range of 1.9 to 2.3 with no significant difference between the treated groups and the control (P=0.54). The apparent digestibility of protein and phosphorus was not affected by the addition of dietary binder (Table 3), nor was there any evidence that lipid digestibility was affected, except in the MV 0.3% treatment. The low lipid digestibility measurement achieved from this treatment was attributable to one value, which was four times outside the standard deviation. This specific sample was reanalyzed a third time but the replicate confirmed the anomalous measurement. Specific growth rate (SGR) and feed conversion ratio (FCR) were not affected by the binder treatments (Table 3).

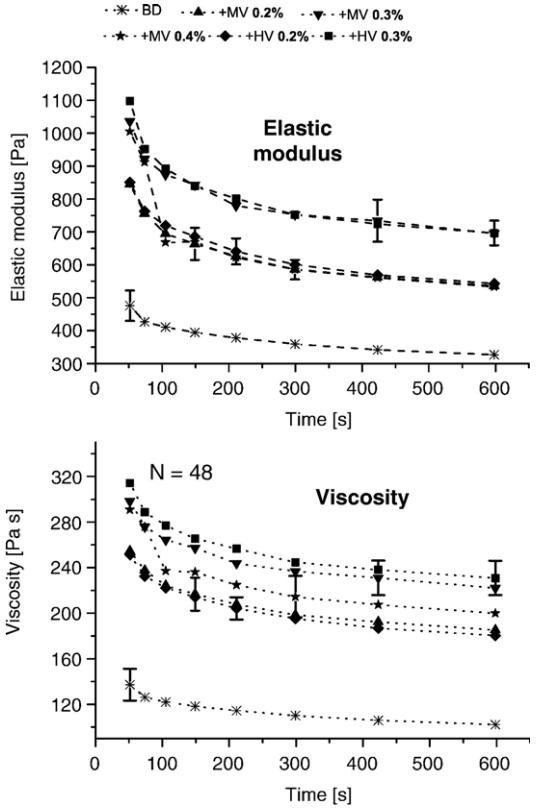

Fig. 2. Viscosity and elastic modulus of trout faeces depending on binder inclusion (mean±average standard error of the mean). (BD=basic diet, MV=mid-viscosity guar gum, HV=high-viscosity guar gum).

Compared with the original feed mix, yttrium oxide levels in the dry faecal matter showed a 3.8-fold increase. Dry matter content was 14.0%±0.12% with no statistically significance difference among the treatments ($P>0.05$).

3.2. Stability of faeces

The addition of various dietary binder treatments resulted in visible differences in texture and structure of dissected faeces, as can be seen in Fig. 1. All faeces containing binder were firmer and more structured than those from the control.

At least two replicates of faecal stability measurements were made for each treatment and tank. The addition of binders improved the viscosity and the elastic modulus of fish faeces significantly in all cases (Fig. 2, Table 4). The effect was significantly positively correlated for the 0.2 and 0.3% inclusion levels (Fig. 2, Table 4). In contrast, the MV 0.4% treatment showed a decreasing effect in terms of the rheological measurements. The effect of the binder was shown to be quality-dependent: with regard to both viscosity and elastic modulus HV yielded a significantly greater improvement than MV ($P<0.0049$).

Table 4
Adjusted mean values for viscosity and elastic modulus of faeces from trout fed the basic diet or the basic diet with added binder

Diet	Rheological properties			
	Viscosity		Elastic modulus	
	Mean	Impr. (%)	Mean	Impr. (%)
Basic diet	72.4^{a} [Pa s]	–	272.6^{a} [Pa]	–
+MV 0.2%	182.0^{b} [Pa s]	+151	542.6^{b} [Pa]	+99
+MV 0.3%	255.6^{c} [Pa s]	+253	843.3^{c} [Pa]	+209
+MV 0.4%	239.6^{c} [Pa s]	+231	694.1^{d} [Pa]	+155
+HV 0.2%	209.3^{b} [Pa s]	+189	670.0^{bd} [Pa]	+146
+HV 0.3%	265.1^{c} [Pa s]	+266	843.2^{c} [Pa]	+209

Means within a column that do not share a common superscript letter are significantly different ($P<0.05$).
Impr. (improvement): percentage improvement compared to basic diet.
(MV=mid-viscosity guar gum, HV=high-viscosity guar gum).

All viscoelastic functions decayed over time due to viscoelastic relaxation (Fig. 2). The progress of this deterioration was comparable for all treatments and seems not to be dependent on binder dose or quality.

The frequency sweep measurements clearly show a shear thinning of all treatments (Fig. 3). These measurements were used to detect differences in the negative slopes of the linear fits because a lower negative slope indicates a greater structural resistance to mechanical stress. According to the statistical analysis (ANCOVA-model: $P<0.00001$, $r^2_{adjusted}=0.960$) there was no significant difference in the slopes ($P>0.97$).

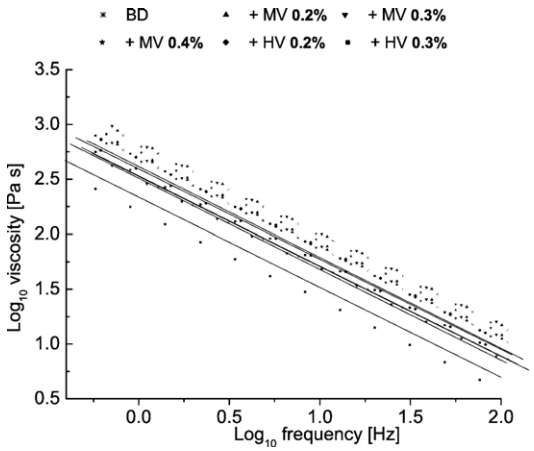

Fig. 3. Frequency sweep data of the rheological measurements including regression lines. Data points of treatments are displaced horizontally by 6%. (BD=basic diet, MV=mid-viscosity guar gum, HV=high-viscosity guar gum).

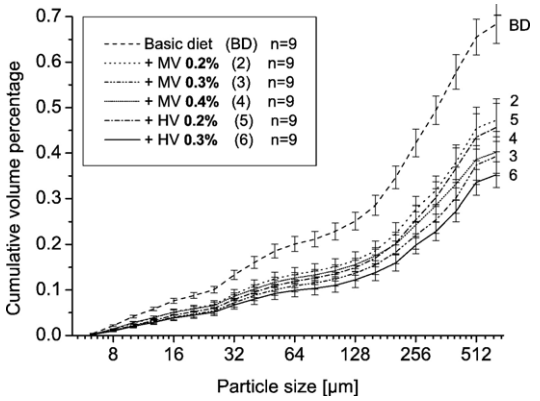

Fig. 4. Volume-dependent cumulative size distributions of suspended particles after breakdown by defined hydro-mechanical stress, depending on binder inclusion (mean±SE). (MV=mid-viscosity guar gum, HV=high-viscosity guar gum).

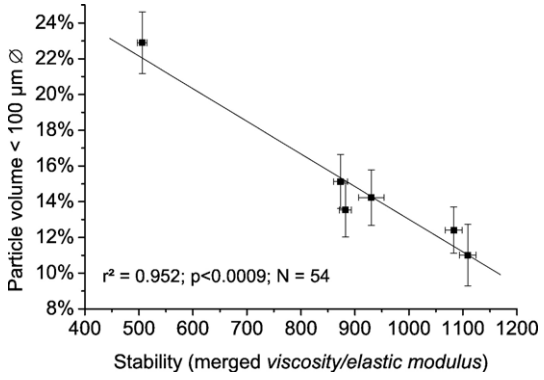

Fig. 5. Dependence of cumulative particle volume below 100 µm on stability of faeces after exposure to a defined hydro-mechanical stress (±SE).

3.3. Particle size distribution

All particle size distributions (PSDs) from faeces of fish fed diets with binders showed a significant ($P<0.05$) shift to larger particles (Fig. 4). The effect increased with the concentration and quality of binder added, with exception of the MV 0.4% (Fig. 4, Table 5). Generally, the effect of the binder was more pronounced with increasing particle size (Fig. 4, Table 5). The difference in cumulative particle size between the basic diet and the HV 0.3% treatment was about 12% at 100 µm and 33% at 600 µm. In accordance with the stability data derived from the rheological measurements, the improvement in particle size parameters achieved through the MV 0.4% treatment was distinctly lower than that for MV 0.3%.

Table 5 shows for each treatment, the percentage of resulting total particle volume that would pass a 100 or 600 µm mesh filter. HV grade gum led to a greater shift towards large particles than MV gum, and thus improves the potential for filtration of suspended solids. HV 0.3% decreased the effluent load that would pass 100 µm by 52% and that passing 600 µm by 48%. With MV 0.3%, the improvement at 100 µm was 46% and at 600 µm it was 43% (Table 5).

The rheological data correlate significantly with the observed PSDs. Plots of the merged values of adjusted means (population marginal means) of viscosity and elastic modulus against the cumulative percentage at 100 µm reveal a highly significant negative linear correlation ($P<0.001$) (Fig. 5). The reduced major axis regression reveals that 95.5% of the observed differences in cumulative particle volume below 100 µm are accounted for by the improved stability of faecal material.

Table 5
Percentages of total particle volume below 100 µm and below 600 µm of suspended faecal particles originating from fish fed the same basic diet but with different binders

Diet	Cumulative particle volume			
	Below 100 µm		Below 600 µm	
	Cum. %	Impr.	Cum. %	Impr.
Basic diet ($n=9$)	22.9	–	68.4	–
+MV 0.2% ($n=9$)	15.1^{s}	+34%	47.2^{s}	+31%
+MV 0.3% ($n=9$)	12.4^{s}	+46%	39.3^{s}	+43%
+MV 0.4% ($n=9$)	14.2^{s}	+38%	40.2^{s}	+41%
+HV 0.2% ($n=9$)	13.6^{s}	+41%	45.6^{s}	+33%
+HV 0.3% ($n=9$)	11.0^{s}	+52%	35.3^{s}	+48%

Cumulative means within a column marked by the superscript s show significant lower values ($P<0.05$) compared to basal diet whereas superscript ns does not.
Impr. (improvement): percentage improvement compared to the control (basic diet without binder) calculated with respect to remaining waste load after filtration at 100 µm and 600 µm. (MV=mid-viscosity guar gum, HV=high-viscosity guar gum).

4. Discussion

Considering that the potential benefits of dietary mediation on physicochemical properties of fish faeces in terms of effluent treatment and husbandry are widely recognised (USDA/CSREES, 1997; Dias and Huelvan, 1998; Ogunkoya et al., 2006) there has been surprisingly little work done within this area (Storebakken, 1985; USDA/CSREES, 1997; Dias and Huelvan, 1998; Amirkolaie et al., 2005b; Ogunkoya et al., 2006). Furthermore, comparisons between the few studies that have been published are made difficult by the lack of consensus regarding the measurement of relevant parameters (i.e. the mechanical property that matters).

For example, previous authors have measured faecal stability indirectly, (e.g. as viscosity in the supernatant of chyme (Leenhouwers et al., 2006)) or directly, as in the present work, or they have measured stability-dependent parameters such as particle size distributions (Han, 1996), faeces recovery by faeces collectors (Amirkolaie et al., 2005a) or image analysis applied to agitated faecal samples (Ogunkoya et al., 2006). Such varied forms of data have very limited use for quantitative comparison.

Matters are made worse still by a lack of reproducible protocols and, in some cases, a lack of appropriate definition of the manipulative agent and its fate during feed production and/or digestion. Without information regarding, for example, the quality of the non-starch polysaccharide (NSP) used, or the nature of its degradation during digestion or feed production, meaningful comparison and/or replication of procedures is near impossible.

The starting point for much of this difficulty, and thus for the different approaches, may be that the concept of 'stability' is not well defined. In the context of managing aquacultural effluents it seems that shear resistance is probably the most relevant stability parameter, as it determines the robustness of faecal particles to turbulence. In well-managed fish farms, this is thought to be the primary cause of undesirable disintegration of faeces into small, and therefore untreatable, particles (Chen et al., 1993; Summerfelt, 1999; Cripps and Bergheim, 2000; Patterson and Watts, 2003). Shear resistance is determined by the adhesiveness (viscosity) and strength (elastic modulus) of the faecal matrix as well as by its structural resistance (shear thinning profile). It is therefore recommended that these properties be measured on those faecal pellets that are on the verge of excretion. These direct physical measurements would be comparable between studies even where other factors such as species, hydrocolloid composition and rearing conditions differ. This is something that would benefit future research in this field greatly.

In all three experimental trials presented here, the fish accepted the experimental diets well and no signs of malnutrition were observed. Binders in general are usually considered harmful to fish, but tangible evidence for this is scarce (Morales et al., 1991; Tacon, 1992). Alginate administered in large quantities (up to 10%) has been shown to cause stomach wall changes in rainbow trout (Storebakken, 1985) and Anderson and Warnick (1964) as well as Maisonnier et al. (2002) observed slight pathological effects, including intestinal damage induced by guar gum in poultry. In the current study however, there was no sign of pathological alteration in the intestine. Some livers exhibited macroscopic alterations. However the frequency (<15%) as well as the intensity of lesions (up to 2.3, indicating incipient to moderate pathology (Bernet et al., 1999, 2004)) were within the normal range for rainbow trout fed high-energy diets (compare positive control: Royal optima®). In general, serious pathological alterations such as necrosis or textural disintegration, were not observed. This was not surprising, given the very low inclusion levels, which prevented the binder attaining an effective concentration in the chyme until the final compaction stages immediately prior to excretion. In the digestibility trial (trial 1) the diet containing the least critical level of guar gum, (mid-viscosity MV 0.2%), was replaced with a commercial product as a positive control with which to test the performance of the binder-free control diet. The apparent digestibility for protein, fat, and phosphorus in both controls were comparable. Furthermore, the introduction of binder to the basic diet was shown to have no significant impact on digestibility in all but one case. Digestibility results for the MV 0.3% treatment showed a significantly reduced lipid digestibility. This surprising result appears anomalous, given that lipid digestibility was not hampered by the higher dose MV 0.4% or by the more effective HV 0.3%. The result also contradicts previous studies in which inclusion of a comparable binder at the same 0.3% concentration produced no significant effect on lipid digestibility (Brinker et al., 2005a). If lipid digestibility were being impaired, the negative effect should be apparent in SGR and FCR data for the trial. No such effect could be shown for any of the binder-added diets, MV 0.3% included. Even Storebakken (1985), in trials using binder concentrations of up to 2.5% of guar gum per dry weight of feed found no significant negative effects on SGR or FCR. Previously, negative effects of guar gum binders on macronutrient digestibility in fish, including rainbow trout, have only been observed when they were added to the diet in extremely high concentrations up to 10 times higher that the current study (Storebakken, 1985; Storebakken and Austreng, 1987; USDA/CSREES, 1997; Carré, 2003). Thus the most likely explanation for the result in this case is an undiscovered sample adulteration effect (perhaps through improper stripping of faeces).

At the macroscopic level, all faeces containing binder are more stable than those without. These findings were confirmed by rheometric data. In all binder-added diets, the viscosity and the elastic modulus of the faeces were improved, and in the case of the most effective treatment, HV 0.3%, the improvement was 266% for

viscosity and 209% for elastic modulus. That elastic modulus shows a less marked improvement than viscosity was not surprising as guar gum forms only synergistic interactions with other hydrocolloids and possesses no true gelation properties (Williams and Phillips, 2002). The improvement was dose-dependent for both grades of binder, with the exception of MV 0.4% in which reduced values for both viscosity and elastic resistance were observed compared to MV 0.3%. Other studies have shown that even very high inclusion levels of guar gum do not increase stability (USDA/CSREES, 1997; Amirkolaie et al., 2005b) in faeces or chyme, for reasons that will be explained later on.

In an aquaculture facility, faeces are continuously exposed to mechanical stress due to water turbulence such as that generated by moving fish or technical installations (Williams and Phillips, 2002; McMillan et al., 2003; Brinker and Rösch, 2005). Thus it is encouraging that the expected decay in viscosity and elastic modulus values with time and repetitive deformations was relatively minor, and more or less parallel for all binder treatments. According to Morris et al. (1981) guar gum solutions are rheologically very stable, and the binder possesses exceptional structural integrity due to continuous re-entanglement of the polysaccharide network. This structural robustness is maintained in real-life situations, hence the effective adhesion of faecal material to form large particles.

The negative slopes of the frequency sweeps indicate shear thinning, i.e. a breakdown of the polysaccharide network due to increasing shear stress. Under such stress, the polysaccharides are disentangled and aligned parallel to the direction of the applied shear stress (Onsoyen et al., 1992). Perhaps surprisingly, the results of the current study provide no evidence that the addition of either mid or high-viscosity binder had any effect on structural resistance, with the slope obtained for the control group similar to that for all treatment groups. An earlier trial by this author and a comprehensive comparison indicated that concentrated all polysaccharide solutions show essentially the same shear thinning profile irrespective of chemical type, molecular weight, solvent environment, or concentration (Morris et al., 1981; Brinker et al., 2005a). It is likely that water-soluble non-starch polysaccharides already present in the basic diet act as a kind of polymeric binder themselves and that these dominate the shear thinning profile of faeces, irrespective of the inclusion of additional polysaccharide binders (Carré, 2003).

Even slight variations in a hydrocolloid concentration, origin, processing or mixture composition can produce substantial changes in the mechanical properties of faeces (Onsoyen et al., 1992). Additionally, the issue is affected by digestive processes such as cellulase activity, fermentation, or changing pH (Hill et al., 1998). Thus it is essential to have data that are reproducible and 'controlled' for these potentially decisive factors. In short, the fate of the stabilising agent (here Guar gum) during feed production and in the gastrointestinal environment of the target species has to be known.

The structure and properties of galactomannans are reviewed by Dea and Morrison (1975). In the context of the current study, the salient properties are the efficiency with which it increases viscosity and its ability to build an interpenetrating network. Guar gum is indigestible to rainbow trout, which lack the enzyme cellulase (Bergot and Breque, 1983; Guillaume et al., 2001). There is, however the possibility of fermentation, if sufficient NSP is available as a substrate for bacterial colonization. Even if such fermentation is not effective enough to degrade the binder completely, it may be able to significantly inhibit its efficacy by physical modifications (Sunvold et al., 1995). Interestingly, higher inclusion levels of NSPs may fail to improve faecal consistency and sometimes even lead to distinctly reduced stability (Storebakken, 1985; Han et al., 1996; Amirkolaie et al., 2005b). Given the close correlation observed between binder concentration and viscosity in particular, this can only be due to some kind of inactivation for which the most evidentiary explanation is microbial fermentation. Possible mechanisms for this include increased faecal moisture content resulting from higher water binding capacity or osmotic pressure from fermentation end products and entrapment of produced gas in faecal strands. Most likely however is a break down of soluble non-starch polysaccharides including those with binding properties (Amirkolaie et al., 2006). This process is suspected to have taken place in the case of the MV 0.4% trial. Most probably not the rather minor increase in substrate for fermentation but an earlier obtained threshold concentration must be the reason. If the binder is active at earlier stages in the gastrointestinal tract it will slow down transport processes and thereby support microbial colonization and degradation by increased exposure time (Choct and Annison, 1992; Choct et al., 1996; Refstie et al., 1999).

The gentle extrudation conditions applied during feed production do not degrade guar gum significantly (Miyazawa and Funazukuri, 2006). The gastrointestinal situation should be comparable for all groups because of the similarity of the basic feed mix and the fact that the binder only becomes effective in the very distal intestine, having already passed the main digestive

processes (Guillaume et al., 2001). This assumption is verified by the accumulation of indigestible yttrium oxide (the concentration of which increased by a factor of ~ 4) and the moisture content of the faecal pellets (about 86%). The final binder concentration values were calculated to be in the range 0.112 to 0.225%, and thus fall within the range of threshold values for useful binding effects (Phillips, 1986).

The presence of dietary binder should result in faeces more resistant to turbulence and thus with a tendency to remain in larger particles. This hypothesis was supported by the size distributions observed in the current study. When un-stabilized (control) and stabilized (binder-treated) faeces were exposed to hydro-mechanical stress, the latter retained significantly larger particles.

The merged rheological measurements of viscosity and elastic modulus correlate significantly with particle size. These stability values accounted for ~ 95% of the improvement in potential solid retention by a 100 μm mesh filter following the deliberate disruption of faeces with a defined shear stress (Cripps and Bergheim, 2000). Clearly, viscosity and elastic modulus are decisive factors in the effective mechanical treatment of trout faeces.

From the smallest to the largest particle size class, the binder-induced consequences on cumulative particle volume increased continuously. This suggests that binder-enhanced resistance to shear is more or less the same across the size spectrum. *Prima facie*, the effect of the binders is most pronounced for large particles. At 600 μm, the cumulative volume difference between the control and most effective treatment HV 0.3% is ~ 33 basal points, whereas at 100 μm this difference is reduced to ~ 11 basal points. However, examination of the effluent after treatment, reveals a rather different picture, with treatment by a 100 μm gauze leading to a reduction in suspended solids of ~ 52%, compared to 48% following treatment by a 600 μm gauze.

The removal of large particles not only reduces overall solid load considerably, it also eliminates an important source of potentially leachable material. In binder-treated faeces, this remains bound up inside large particles wherein exposure to water in which it might be dissolved and become unrecoverable is minimized. Thus water quality is significantly improved and mechanical waste removal is optimized (Brinker et al., 2005a).

Similar improvements in removal efficiency could in theory be achieved with the use of smaller filter mesh sizes to allow the removal of smaller solids. But the resulting increase in hydraulic loading and backwash effects would also increase investment and operational costs exponentially (Summerfelt, 1999; Cripps and Bergheim, 2000). The same drawback applies to improvements in sedimentation and hydraulic residence times (Henderson and Bromage, 1988; Bergheim et al., 1998; Engle and Valderrama, 2003).

With respect to current market prices and effectiveness, HV costs of 37.5% or 0.75 € more per kg than MV, and reduces post-filtration solid load by an additional 6% (equivalent to an extra removal of 14.4–19.1 kg suspended solids per metric ton of fish produced (Bureau et al., 2003)). Given the low inclusion concentrations required for effective binding, the overall cost is only 2.25 € per ton of feed, an investment that appears well justified.

5. Conclusions

The results obtained in this study support earlier work showing that the addition of dietary binders to fish feed significantly enhances the stability of faeces and thus promotes the formation of large waste particles with high mechanical removal potential. This effect occurs in a dose- and quality-dependent manner but with an upper threshold for improvement. The positive results occurred without negative side effects on the health of the fish and without affecting efficacy of the diets.

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