

Comparison of hydrolysed proteins from different raw materials in diets for Mozambique tilapia *Oreochromis mossambicus*

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Abstract The study aimed to compare three fish protein hydrolysates (FPH) from different origins as dietary ingredients for Mozambique tilapia *Oreochromis mossambicus*. The ingredients were self-prepared fish silage from rainbow trout viscera and two commercial FPH products from shrimp and mixed marine by-products. Each ingredient was further included at two dietary levels: a low level where hydrolysed protein contributed 20 g kg⁻¹ protein to the diet and a high level where protein contribution was 40 g kg⁻¹. It was found that all three FPH products sustained high specific growth rates in excess of 2.5 % of body weight per day at both inclusion levels and that there were no negative impacts on production performance, animal health or intestinal gross morphology compared to the control. Further, there were no significant differences in specific growth rate, feed conversion, dress-out %, non-specific immunity parameters or intestinal morphology between any treatments. However, low inclusion of FPH from mixed marine origin (treatment HCL) showed significantly higher growth than high inclusion of self-prepared silage (treatment SH) through significantly higher final animal length and height; a simultaneous significant decrease in Jones condition factor in treatment HCL might be explained by a change in animal body composition. It is concluded that all FPH products were acceptable feed ingredients at the inclusion levels investigated; however, differences in animal growth seen between treatments HCL and SH might be the result of the different inclusion levels employed, and the FPH raw material origin. Further, interpretation of animal condition factor should also take into account supplementary trial data, as differences between treatments might not signify differences in animal well-being, but rather changes in body composition.

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Abbreviations

ANOVA	Analysis of variance
FCR	Feed conversion ratio
FPH	Fish protein hydrolysate
<i>H</i>	Height
IG	Serum immunoglobulin
Jones CF	Jones condition factor
<i>L</i>	Length
mg	Milligram
ml	Millilitre
SE	Standard error of the mean
SGR	Specific growth rate
<i>t</i>	Time
TP	Serum total protein
<i>W</i>	Weight
Wid	Width
μg	Microgram

Introduction

Fish protein hydrolysates (FPH) from fish and shellfish origin have been proposed as partial or total substitutes for fishmeal as protein source in aquaculture diets for finfish (Aksnes et al. 2006b; El-Sayed 1998; Nwanna et al. 2004; Ramasubburayan et al. 2013; Soltan et al. 2008). When produced from processing by-products, FPH can contribute to the sustainability of the aquaculture sector through decreased reliance on wild caught fish for sourcing of feed ingredients (Naylor et al. 2009). Apart from acting as protein source, some FPH exhibit additional beneficial functional or bio-active effects when incorporated in formulated diets, e.g. increased intestinal enzyme activity, improved disease resistance and survival, stimulation of the non-specific immunity, increased levels of insulin-like growth factor in blood plasma and stimulation of feed intake (Bui et al. 2014; Cahu 1999; Chotikachinda et al. 2013; Kotzamanis et al. 2007; Kousoulaki et al. 2013; Liang et al. 2006; Tang et al. 2008; Zheng et al. 2012). Additionally, improved growth performance upon dietary FPH inclusion has also been widely reported in various finfish species (Bui et al. 2014; Goncalves et al. 1989; Refstie et al. 2004; Zheng et al. 2012, 2013).

FPH products have also been evaluated specifically in tilapia species. Tilapias are a globally important group of aquaculture species, and significant amounts of fishmeal are used to produce formulated feeds for them (Bostock et al. 2010; FAO 2010). As in other finfish, FPH products have been evaluated as protein source and potential replacers of fishmeal (Cavalheiro et al. 2007; El-Hakim et al. 2007; Fagbenro et al. 1994; Leal et al. 2010; Plascencia-Jatomea et al. 2002) and soybean meal (Hernández et al. 2013), as immune stimulant (Goosen et al. 2014a) or to determine the effects of FPH inclusion on intestinal digestive enzyme activity (Santos et al. 2013). In fishmeal replacement studies, it

was generally found that FPH can replace a portion of the fishmeal in formulated tilapia diets without negatively impacting production performance.

FPH are produced from a range of raw materials, and currently a number of different commercial FPH products are available for inclusion in aquafeeds; however, comparative studies where FPH from different origins are evaluated simultaneously are lacking (Bui et al. 2014). As different FPH products differ as a result of different raw materials and production processes (Kristinsson and Rasco 2000a, b), comparative studies assist in the proper selection of optimal feed ingredients. Feed ingredients, however, should not only be selected for the ability to provide good animal growth, but also for their ability to ensure good animal health status; it is therefore necessary to ensure that dietary FPH inclusion does not cause decreased health status (e.g. depressed immune function) in experimental animals (Kiron 2012).

The aim of the study was therefore to do a comparative growth study where three different FPH products were evaluated (each at two dietary inclusion levels) for effects on production performance. Any effects on health status of the FPH products were monitored by way of blood and non-specific immunity parameters and liver weight, while effects on the digestive tract were monitored by way of the gross intestinal morphology.

Materials and methods

Experimental design, system and animals

Three different hydrolysed protein products were evaluated at two dietary inclusion levels in a feeding trial and compared to a control diet, resulting in a total of seven treatments. The three hydrolysates used in the trial were rainbow trout viscera silage (self-prepared), a commercial shrimp by-product hydrolysate (Actipal HP1, Aquativ, France) and a commercial hydrolysate from mixed marine by-products (Actipal HC2, Aquativ, France). The diets were formulated such that the protein contribution in the low inclusion diets amounted to 20 g kg⁻¹ final feed while the high inclusion diets contained 40 g kg⁻¹ protein from the protein hydrolysates. Diets were formulated to be iso-proteic and iso-energetic (refer to Table 1). The high inclusion level of silage was determined from a previous investigation using rainbow trout silage and *Oreochromis mossambicus* (Goosen et al. 2014a), where 285 g wet silage per kg feed was shown to inhibit animal growth, and this inclusion level was therefore taken to be the maximum for the current trial.

Six replicates were used per treatment, with 15 fish initially stocked per tank (90 fish per treatment). The experimental animals were sex-reversed Mozambique tilapia *O. mossambicus* from a single cohort, with a mean initial weight ranging from 10.3 to 11.0 g, with no significant differences between the respective treatments (refer to Table 2). The experimental system used was a heated, indoor recirculating system at Welgevallen Experimental Farm, at Stellenbosch University. The volume of experimental tanks was 80 litres, and continuous aeration was supplied. Water temperatures were 26–30 °C for the duration of the trial. Fish were fed three equal portions daily at approximately 3 % of body weight per day.

As indicators of production performance, fish growth rate, feed conversion, animal weight, length, height, the Jones condition factor (Jones CF) and the dress-out % at final sampling were determined. Fish health status was characterised as a combination of non-specific immunity parameters (serum lysozyme, total protein and immunoglobulin concentration), red blood cell volume (haematocrit values) and the hepatosomatic index (liver

weight as a percentage of body weight). The intestinal gross morphology of the mid-intestine was monitored as an indication of intestinal changes that might result from the different dietary ingredients.

The feeding trial was run for a duration of 61 days and had two samplings: Sampling 1 (34 days after trial initiation) and Sampling 2 (at the conclusion of the trial). At Sampling 1, all fish in each tank were weighed and measured, and blood was withdrawn from one randomly chosen animal per tank to determine non-specific immunity parameters and red blood cell volume. Sampled animals were removed from the experimental tanks and were not replaced. At Sampling 2, the same parameters were measured as at Sampling 1; additionally, the fish sampled for measuring blood parameters were slaughtered to determine dress-out % and liver weight, while the same animals' intestinal tracts were used for the determination of gross intestinal morphology.

Diet preparation and analysis

Rainbow trout silage was prepared and de-oiled as described previously (Goosen et al. 2014b, c). A commercial tilapia diet using fishmeal and soybean meal as main protein sources (AquaNutro Finfish Starter, NutroScience, Malmesbury, South Africa, proprietary formulation) was used as basal diet for all treatments. Diets were prepared by mixing all ingredients with the basal diet and a specified amount of water to form moist paste suitable for extrusion; diets were subsequently extruded at 70 °C and dried in a ventilated drying oven at a temperature of 55 °C. The amount of water added prior to extrusion was adjusted to take into account the water content of the various protein hydrolysates. The proximate composition of the feeds was determined using standard AOAC analyses (AOAC 2003) (refer to Table 1).

Table 1 Formulations and proximate composition of experimental diets

Ingredient (g kg ⁻¹)	SL	SH	HPL	HPH	HCL	HCH	C
Diet formulations							
Basal diet	805	712	940	940	902	876	940
Fishmeal	30	0	30	0	38	9	60
Trout silage ^a	165	288	–	–	–	–	–
HP ^b	–	–	30	60	–	–	–
HC ^c	–	–	–	–	60	115	–
Proximate composition (g kg ⁻¹)							
Crude protein	471	472	474	466	474	470	462
Crude fat	64	59	61	62	62	60	59
Moisture	59	62	54	54	54	56	59
Ash	112	109	112	116	116	115	115
Carbohydrates ^d	294	298	299	302	294	299	305
Gross energy (MJ kg ⁻¹)	18.7	18.6	18.8	18.7	18.7	18.7	18.5

^a Crude protein—103 g kg⁻¹; crude fat—19 g kg⁻¹; ash—7 g kg⁻¹; moisture—862 g kg⁻¹

^b Crude protein—620 g kg⁻¹; crude fat—120 g kg⁻¹; ash—140 g kg⁻¹; moisture—60 g kg⁻¹

^c Crude protein—320 g kg⁻¹; crude fat—50 g kg⁻¹; ash—80 g kg⁻¹; moisture—520 g kg⁻¹

^d Determined by difference

Experimental and analytical procedures

Animals were only handled after being anaesthetised with AQUI-S (Lower Hutt, New Zealand). All experimental and analytical procedures were approved by Stellenbosch University Ethics Committee (certificate number SU_ACUM_00018).

Production performance parameters

Production performance was characterised by computing specific growth rate (SGR), feed conversion ratio (FCR) and the Jones CF. The respective formulae used were $SGR = 100 \times [\ln(W_f/W_i)]/(t)$, with units of grams and days for W and t , where W_f denotes the final mean animal weight and W_i initial mean weight; $FCR = (W_f - W_i)/\text{feed given}$, with animal weight and feed given in units of grams; and Jones CF = $1,000 \times W/L^2H$, with W in grams and L (animal length) and H (animal height) in units of cm, according to Jones et al. (1999). Dress-out % is defined as the carcass weight after animals were gutted and gilled and was expressed as the percentage of initial weight.

Animal health status

At each sampling, blood was collected from the caudal veins of sampled animals for determination of non-specific immunity parameters, using sterile 27-gauge syringes. The blood serum was recovered for the determination of lysozyme, total protein and immunoglobulin concentration, as described previously (Goosen et al. 2014c). A portion of the blood was put into heparin-treated Eppendorf tubes and used to determine the haematocrit values using glass capillaries in a haematocrit centrifuge. At Sampling 2, the liver weight of each slaughtered animal was recorded to determine the hepatosomatic index.

Intestinal morphology

Gross intestinal morphology of the mid-intestine of experimental animals was monitored according to the methods described previously (Goosen et al. 2014c).

Statistical analysis

All data were analysed by way of one-way ANOVA, with a significance level of $P < 0.05$. Tukey's post hoc test was applied to determine which means differed if ANOVA indicated the existence of significant differences between means. All data analysis was performed with the Statistica software package (version 11, StatSoft, Inc.).

Results

Production performance

All diets were accepted readily by the experimental animals, and no mortalities occurred during the trial. Production performance parameters as evaluated at the end of the trial (Sampling 2) are summarised in Table 2 and Fig. 1. SGR values ranged from $2.59 \pm 0.14 \text{ day}^{-1}$ (mean \pm standard error) for treatment SH to $2.84 \pm 0.11 \text{ day}^{-1}$ in treatment HCL; however, there were no statistically significant differences between any

Table 2 Summary of production performance parameters

	SL	SH	HPL	HPH	HCL	HCH	C
W_1 (g)	10.3 ± 0.26	10.9 ± 0.35	11.0 ± 0.29	10.7 ± 0.30	10.7 ± 0.33	11.0 ± 0.33	11.0 ± 0.36
SGR (day^{-1})	2.81 ± 0.08	2.59 ± 0.14	2.66 ± 0.12	2.71 ± 0.08	2.84 ± 0.11	2.67 ± 0.13	2.67 ± 0.13
FCR	1.35 ± 0.04	1.60 ± 0.23	1.42 ± 0.07	1.37 ± 0.04	1.22 ± 0.05	1.51 ± 0.11	1.47 ± 0.08
Dress-out %	82.5 ± 1.07	81.9 ± 0.80	82.7 ± 0.62	83.2 ± 1.20	82.9 ± 0.81	82.6 ± 0.76	78.8 ± 3.88

Data are presented as mean ± standard error (SE)

W_1 , Initial weight

treatments for SGR. There were also no statistically significant differences in FCR, dress-out % (Table 2) or final animal weight (Fig. 1b) between any treatments. The highest FCR value was 1.60 ± 0.23 for treatment SH, while the lowest was seen in treatment HCL (1.22 ± 0.05). Dress-out % and final animal weight of all treatments were between 78.8–82.9 % and 53.7–60.9 g, respectively.

There were no significant differences between any of the treatments and the control for final animal length, height or Jones CF (Fig. 1). Treatment HCL had significantly higher animal length and height than treatment SH (5.1 and 6.8 % increase in length and height, respectively), while Jones CF for treatment HCL was significantly lower than that of treatment SL (by 4.9 %) and SH (by 4.0 %). There were no significant differences in any of the production performance parameters or animal measurements at Sampling 1 (data not shown).

Animal health status

Table 3 reports the different parameters used to evaluate the health status of the animals. There were no statistically significant differences at Sampling 1 or Sampling 2 between any of the treatments for any of the parameters evaluated. At Sampling 1, serum lysozyme concentration was between 16.7 and 27.4 $\mu\text{g ml}^{-1}$, total protein concentrations between 20.4 and 21.8 mg ml^{-1} , immunoglobulin levels 1.34–3.21 mg ml^{-1} and haematocrit values between 39.9 and 43.3 %. The liver weights of animals were not evaluated at Sampling 1. At Sampling 2, lysozyme concentrations were 14.5–20.9 $\mu\text{g ml}^{-1}$, total protein 21.7–23.2 mg ml^{-1} , immunoglobulin 1.87–2.47 mg ml^{-1} , haematocrit values

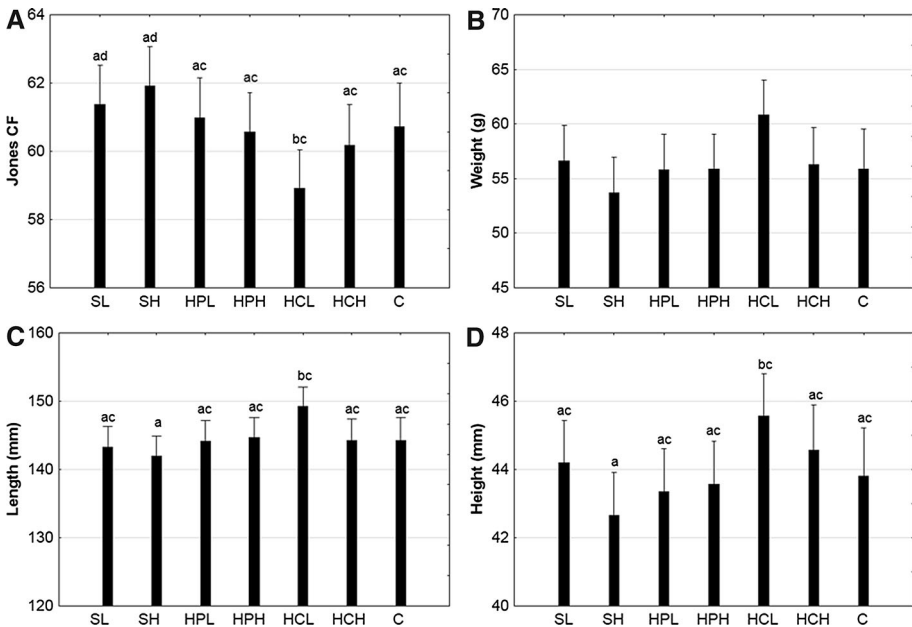


Fig. 1 Jones CF (a), animal weight (b), length (c) and height (d) as measured at the conclusion of the trial. Values are shown as mean \pm 95 % confidence intervals. Common text superscripts indicate that means do not differ significantly

Table 3 Indicators of fish health and immunity

	SL	SH	HPL	HPH	HCL	HCH	C
Sampling 1							
Lysozyme ($\mu\text{g ml}^{-1}$)	20.6 \pm 2.78	25.3 \pm 4.35	27.4 \pm 6.58	19.8 \pm 3.8	22.4 \pm 3.70	16.7 \pm 2.60	17.6 \pm 2.52
TP (mg ml^{-1})	20.4 \pm 0.94	20.4 \pm 0.25	21.5 \pm 1.53	21.5 \pm 1.15	21.8 \pm 1.29	21.0 \pm 0.97	21.0 \pm 1.14
IG (mg ml^{-1})	1.73 \pm 0.94	1.34 \pm 0.56	3.21 \pm 1.10	1.75 \pm 0.70	1.55 \pm 0.54	2.11 \pm 0.80	3.00 \pm 1.35
Haematocrit (%)	41.4 \pm 1.55	43.3 \pm 2.47	41.7 \pm 1.08	41.1 \pm 1.32	40.1 \pm 1.58	41.0 \pm 2.17	39.9 \pm 1.44
Sampling 2							
Lysozyme ($\mu\text{g ml}^{-1}$)	14.5 \pm 2.46	20.9 \pm 4.30	20.1 \pm 3.30	16.9 \pm 2.20	19.5 \pm 3.09	18.5 \pm 2.21	16.9 \pm 2.81
TP (mg ml^{-1})	22.2 \pm 0.51	23.2 \pm 0.19	22.2 \pm 0.46	22.7 \pm 0.82	21.9 \pm 0.31	21.7 \pm 0.60	23.1 \pm 0.38
IG (mg ml^{-1})	1.87 \pm 0.45	2.13 \pm 0.24	1.96 \pm 0.52	2.45 \pm 0.58	2.47 \pm 0.30	1.94 \pm 0.78	2.05 \pm 0.41
Haematocrit (%)	41.3 \pm 1.76	42.8 \pm 2.87	41.3 \pm 1.20	41.8 \pm 1.33	40.0 \pm 1.86	41.5 \pm 2.50	40.6 \pm 1.50
Hepatosomatic index (%)	2.13 \pm 0.27	2.27 \pm 0.25	2.08 \pm 0.17	2.19 \pm 0.25	1.95 \pm 0.18	2.25 \pm 0.14	1.96 \pm 0.25

Data are presented as mean \pm SE

TP total protein, IG immunoglobulin

40.0–42.8 % and hepatosomatic index values 1.95–2.27 %. Visual inspection of internal organs revealed no apparent differences (e.g. abnormally coloured livers or intestinal fat build-up) between treatments that warranted further investigation.

Intestinal morphology

Measurements of the gross intestinal structure for animals in the different treatments are presented in Table 4. There were no statistically significant differences between any of the measured gross morphological features. The values of mean inner perimeter length of intestines were between 9,731 and 11,151 μm , mean outer perimeter 5,356 and 6,290 μm , mean fold length 129 and 165 μm , mean fold width 102 and 117 μm , mean mucosa thickness 29.0 and 43.3 μm and the ratio of fold length to fold width ($L:Wid$) ranged from 1.30 to 1.68.

Discussion

Commercial FPH products from a variety of raw material origins are available for feed formulation, including hydrolysates from krill, marine finfish by-products and shrimp processing by-products. Nutritional analysis provides essential information regarding the suitability of feed ingredients for dietary inclusion for particular species. However, when ingredients have similar nutritional profiles, comparative studies that evaluate ingredients simultaneously under the same conditions are required to select the optimal ingredient. Therefore, the current study investigated three FPH products and monitored effects on animal growth, health and intestinal morphology.

All three FPH products served as good feed ingredients at the inclusion levels evaluated and sustained high growth rates in excess of 2.5 % increase in bodyweight per day (Table 2). The various FPH products contributed to the protein content of the experimental diets and successfully substituted a portion of fishmeal. In studies with Nile tilapia (*Oreochromis niloticus*), total replacement of fishmeal was achieved with fish and shrimp head silage without significant negative impacts of growth (Cavalheiro et al. 2007; El-Hakim et al. 2007); however, in another study, shrimp head silage replacing more than 15 % of fishmeal leads to significantly decreased growth (Plascencia-Jatomea et al. 2002), while shrimp silage included at 6 % of the final diet (corresponding to 20 % fishmeal replacement) had no significant reduction in production performance (Leal et al. 2010). In the current trial, total FPH inclusion was restricted, as previous studies using rainbow trout silage in diets for *O. mossambicus* showed growth depression at dietary protein contribution of 40 g kg^{-1} (Goosen et al. 2014a). Due to the restricted inclusion level of silage and the experimental design where each of the FPH was to contribute equivalent levels of protein to the final diet, the maximum inclusion level of the FPH products was not established in the current trial.

The trial showed that the combination of FPH type and dietary inclusion level can significantly affect production performance parameters. The low inclusion level of mixed-origin hydrolysate (HCL) resulted in fish with significantly higher total animal length and height compared to the high silage treatment (SH) and can therefore be said to have exhibit better growth (Fig. 1). Final animal weights and SGR in treatment HCL were also higher than in treatment SH, but differences were not significant. However, the differences might have become statistically significant if the trial was run for a longer period which more closely represents a full grow-out cycle. The reason for higher growth in treatment HCL

Table 4 Summary of data for intestinal morphology (mean \pm SE)

	SL	SH	HPL	HPH	HCL	HCH	C
Inner perimeter	9,731 \pm 958	10,150 \pm 717	10,524 \pm 1,273	10,704 \pm 1,027	11,151 \pm 1,296	10,570 \pm 1,447	11,234 \pm 1,428
Outer perimeter	5,932 \pm 533	5,757 \pm 422	5,590 \pm 668	6,198 \pm 377	5,764 \pm 266	5,356 \pm 271	6,290 \pm 589
Villi length	147 \pm 17.8	144 \pm 12.8	154 \pm 24.0	139 \pm 9.30	165 \pm 24.2	160 \pm 20.7	129 \pm 17.6
Villi width	110 \pm 8.75	113 \pm 6.83	117 \pm 10.1	104 \pm 8.65	112 \pm 11.2	108 \pm 19.0	102 \pm 18.4
L:Wid	1.38 \pm 0.21	1.30 \pm 0.13	1.34 \pm 0.22	1.35 \pm 0.08	1.50 \pm 0.16	1.68 \pm 0.32	1.31 \pm 0.09
Mucosa width	39.5 \pm 4.16	34.5 \pm 2.33	43.3 \pm 7.00	29.0 \pm 4.23	33.0 \pm 4.32	41.5 \pm 8.85	37.4 \pm 3.52

All values are reported in units of μm , except the L:Wid which is a dimensionless quantity

L:Wid ratio of villi length to villi width

than SH might be because the high silage inclusion was affecting growth rate. An equivalent silage inclusion level has been shown in a previous trial to negatively impact growth in *O. mossambicus* (Goosen et al. 2014a). In a comparative FPH evaluating three hydrolysates from different origins at a single inclusion level in red seabream, it could not be shown that there were significant differences in production performance (Bui et al. 2014); however, only two of the ingredients significantly improved performance compared to the control.

There were no indications that the FPH products had any negative impacts on animal health status or intestinal morphology. Humoral non-specific immune parameters and haematocrit values were measured at both samplings and served as indicators of health status at the two different sampling times. The lack of significant differences between treatments serves as indication that the different dietary ingredients did not affect animal health negatively. Further, at Sampling 2, neither the hepatosomatic index or gross intestinal morphology differed significantly between treatments, further indicating that dietary FPH supplementation had no negative impacts on fish health or the digestive system. FPH also had no effect on hepatosomatic index in Atlantic salmon (Espe et al. 2012). It is known that dietary FPH inclusion can result in increased levels of some humoral immune parameters in various finfish species (Bui et al. 2014; Liang et al. 2006; Tang et al. 2008); however, no increases were seen in the current trial. Increased phagocytic activity (a measure of cellular non-specific immunity) has been reported before in *O. mossambicus* upon dietary fish silage supplementation, but effects on cellular immunity were not investigated in the current trial.

Caution should be used when interpreting differences in fish condition as measured with the use of condition factors. The significantly lower Jones CF found in treatment HCL when compared to both silage containing treatments indicates that there were significant differences in the relative bodily proportions of the animals in the two treatments. However, the practical significance of these differences is difficult to interpret in the present trial. The conventional interpretation is that fish with a higher condition factor are in better physiological condition and general health (Jones et al. 1999; Richter et al. 2000); however, it is acknowledged that the relationship between condition factor and the physiological status of fish is not always clear (Sutton et al. 2000). Applying the conventional interpretation to the data of the current trial, the conclusion might be reached that fish in treatment HCL were in worse condition than those in treatments SL and SH, a conclusion which is clearly not true from growth parameter and immunity data. In fact, fish in treatment HCL were significantly longer and higher than those in treatment SH and had numerically higher final weights than both silage containing diets. Conclusions of the trial outcome should therefore not be based on condition factor data alone, but should also take into account the additional data gathered.

The decrease in Jones CF seen for treatment HCL compared to treatments SL and SH could be explained by a change in body composition as a result of FPH supplementation. An alternative explanation for the change in condition factor that is linked to changes in body composition is discussed by Richter et al. (2000). The various condition factors are essentially a calculation of fish density from animal weight and a combination of animal length and height, depending on the specific condition factor. In the case of the Jones CF, the conventional interpretation where fish condition increases with increasing condition factors is applied by the authors (Jones et al. 1999). However, in the case where condition factor only varies according to fish density, an increase in fish condition should decrease the condition factor as water is replaced by less dense components like fat and protein in the body with a fairly constant amount of ash (Richter et al. 2000). In hydrolysed protein

studies, significant changes in carcass composition were found in Nile tilapia (El-Hakim et al. 2007; Leal et al. 2010), rainbow trout (Aksnes et al. 2006a) and carp larvae (Carvalho et al. 1997) upon dietary supplementation of different levels, size-fractions and types of protein hydrolysates. The effect of FPH supplementation on body composition was not studied in the current trial.

Conclusion

Growth performance indicated that all protein hydrolysates evaluated were good feed ingredients for total protein contribution levels from the hydrolysates as high as 40 g kg⁻¹. However, growth performance of treatments was not equivalent and might be a result of hydrolysate raw material origin and dietary inclusion level. There were further no negative impacts on animal health status or gross intestinal morphology from any of the treatments. The results obtained for the Jones CF highlight the possible difficulties in the interpretation of condition factor data when there are significant differences between treatments; it is recommended that conclusions not be made based on condition factor data alone, but that supplementary trial data should also be taken into consideration.

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