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The effects of protein hydrolysates on the immunity and growth of the abalone *Haliotis midae*

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ABSTRACT

Dietary hydrolysed proteins have been shown to stimulate the non-specific immunity of various finfish species, whilst the potential for immune stimulation of these feed ingredients in abalone has not been investigated. The immune-stimulating potential of two hydrolysed protein sources (self-prepared fish silage and a commercial fish protein hydrolysate) at two dietary inclusion levels in abalone diets was therefore measured in the South African abalone *Haliotis midae*, using animals of initial mean weight of 123 g–128 g. Diets containing the high inclusion levels were also fed in two feeding regimes: continuous feeding, or phase feeding, where the hydrolysed protein diets were alternated monthly with the control diet. In low inclusion diets, hydrolysate inclusion contributed 6 $g \cdot kg^{-1}$ protein in the final diet, whilst in high inclusion diets this was increased to 18 $g \cdot kg^{-1}$. It was found that the low inclusion level of the commercial hydrolysate significantly increased the cellular immunity through increasing the phagocytic activity of haemocytes by 18% compared to the control diet, whilst none of the other diets showed any significant differences compared to the control. Both inclusion levels of the commercial hydrolysed protein significantly improved daily weight increase of animals. Phase feeding had no positive impacts on immunity or production performance. The inclusion of both types of hydrolysed protein sources significantly decreased the water stability of feeds compared to the control, except at the lowest inclusion level of the commercial hydrolysate. It is concluded that the use of dietary hydrolysed proteins can lead to improved cellular immunity and growth in abalone, however it is important to determine appropriate inclusion levels to prevent negative impacts on feed water stability and production performance.

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1. Introduction

The potential benefits to abalone derived from the functional and bio-active properties of enzymatically hydrolysed proteins included in diets are not well described. In *Haliotis fulgens*, dietary inclusion of autolysed abalone viscera increased feed attraction (Viana et al., 1994) and successfully served as dietary protein source and fishmeal replacement (Guzmán and Viana, 1998; Viana et al., 1996). The use of hydrolysed proteins in finfish diets has been reported widely, where both autolysed proteins (fish silage) and protein hydrolysates prepared with added enzymes have been employed successfully in aquafeeds, with improved production performance reported in eel, African catfish, Atlantic salmon, large yellow croaker and red seabream (Bui et al., 2014; Goncalves et al., 1989; Refstie et al., 2004; Soltan et al., 2008; Tang et al., 2008). The reported biological effects included the improvement of disease resistance and survival in European sea bass larvae (Cahu, 1999; Kotzamanis et al., 2007), stimulation of the non-specific immunity in Japanese sea bass, large yellow croaker and red seabream (Bui et al., 2014; Liang et al., 2006; Tang et al., 2008), increased intestinal enzyme activity in European sea bass larvae (Kotzamanis et al., 2007) and an alteration of the spatial gene expression of a possible di- and tri-peptide transporter in Atlantic cod (Bakke et al., 2010).

Hydrolysed dietary proteins have been linked to stimulation of the non-specific immunity in finfish (Bui et al., 2014; Liang et al., 2006; Tang et al., 2008), although not reported in abalone. Improved immunity in abalone could have benefits to producers, as a good immune response is critically important in ensuring good disease resistance (Gopalakrishnan et al., 2009; Hooper et al., 2007). However, continuous feeding of dietary immune stimulants to slow-growing abalone could be counterproductive and increase production costs, as is known in finfish where continual feeding of immune stimulants over an extended period of time can lead to decreased efficacy of immune stimulants (Dr. Galina Jeney, personal communication). Further, in abalone it has also been hypothesized that over-stimulation of immune functions could lead to impaired growth (Hooper et al., 2010).

Suitable inclusion levels of hydrolysed proteins need to be established in abalone diets, as high inclusion of hydrolysed fish proteins in finfish diets can lead to decreased growth, presumably as a result of excessive

Abbreviations: DOH, degree of protein hydrolysis; LSD, least significant difference; DWG, daily weight gain; FCR, feed conversion ratio; RFI, relative feed intake.

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amounts of free amino acids and short chain peptides (Espe et al., 1992). Decreased growth upon high inclusion of hydrolysed protein has been reported in African catfish (Soltan et al., 2008), rainbow trout (Guzel et al., 2011), carp fingerlings (Ramasubburayan et al., 2013) and sea bass larvae (Cahu, 1999; Kotzamanis et al., 2007). The threshold where dietary inclusion level of hydrolysed fish proteins in abalone diets that cause decreased growth has not been reported.

In addition to inclusion levels of hydrolysed proteins in abalone diets, the potential impact on water stability of feeds also requires consideration. Abalone feeds must maintain water stability during long periods of submerging, due to slow feeding habits of abalone. Leaching of soluble ingredients or physical disintegration of feed will result in direct economic losses and may have adverse impacts on water quality (Kirkendale et al., 2010). However, the water solubility of hydrolysed fish proteins is higher than for the corresponding intact proteins (Kristinsson and Rasco, 2000) and inclusion of hydrolysed proteins could therefore compromise the water stability of feeds (Guzmán and Viana, 1998).

The study aimed to evaluate the effect of two hydrolysed protein products on immune function and production parameters of South African abalone *Haliotis midae*, at two dietary inclusion levels and using different feeding regimes, and to determine the effects of hydrolysed proteins on the water stability of abalone feeds.

2. Materials and methods

2.1. Experimental design

The experiment consisted of seven treatments (refer to Table 1), each replicated 6 times. The treatments comprised of a control diet (C) that was prepared using a basal diet (AquaNutro Abalone Grower, NutroScience, Malmesbury, South Africa) supplemented with 25 $g \cdot kg^{-1}$ trout silage oil, two diets with a low (SL) and high (SH) level of self-prepared wet rainbow trout viscera silage (endogenous enzyme hydrolysate), and two diets with a low (HPL) and high (HPH) level of a commercially available hydrolysate (enzymatically hydrolysed product; ACTIPAL HP 1, AQUATIV, France) — all fed continuously for the duration of the 153 day experimental period. The diets containing the higher levels of silage (SH) and hydrolysate (HPH) were duplicated in two treatments that employed alternating feeding regimes (30-day intervals) with the control diet. As the current trial formed part of a larger investigation, the control diet had to be supplemented with trout silage oil in order to achieve iso-nutrient formulations over the entire investigation.

Inclusion level of fish silage proteins was determined such that the crude protein component of the fish silage comprised 6 $g \cdot kg^{-1}$ of the final feed (after drying) for the low inclusion level, and 18 $g \cdot kg^{-1}$ for the high inclusion level. Feeds in which the commercial protein hydrolysate were incorporated, were formulated to be iso-proteic and iso-energetic to the corresponding silage diets. See Table 2 for diet formulations and proximate composition.

2.2. Ingredient and diet preparation

Fish silage was prepared from rainbow trout viscera as described previously (Goosen et al., 2014). Floating oil was recovered from the silage after 3 days through manual decantation and antioxidant (1 $ml \cdot l^{-1}$ butylated hydroxytoluene and butylated hydroxyanisole mixture, Oxipet L, Bitek) was added. Recovered oil was stored in airtight plastic containers at 4 °C and utilised in diet preparation 12 days post-silage preparation. Silage was de-oiled with a centrifugal dairy separator (Elecrem Model 1, Elecrem, France). The degree of protein hydrolysis (DOH) in the silage was calculated using the trichloroacetic acid (TCA) precipitation method of Hoyle and Merrit (1994). Equal volumes of a 20% (w/v) TCA solution in distilled water and de-oiled silage were mixed at room temperature and incubated for 1 h. The mixtures were centrifuged for 5 min at 14,000 rpm, supernatant was sampled and analysed for total nitrogen using the Kjeldahl method (AOAC, 2003). The DOH value was expressed as the percentage of non-precipitated nitrogen to total sample nitrogen and was found to be 90.9%.

Table 1
Summary of experimental diets and the combination of diets fed in each treatment.

Diet/ingredient	Inclusion level	Feeding regime	Diets fed	Treatment
Reference	N/A	Constant	C	C
Rainbow trout silage	Low	Constant	SL	SL
	High	Constant	SH	SH
	High	Phase	SH + C	SH, Phase
Commercial hydrolysate	Low	Constant	HPL	HPL
	High	Constant	HPH	HPH
	High	Phase	HPH + C	HPH, Phase

To prepare the diets, the two hydrolysed proteins were mixed with the oil and basal diet and a specified amount of water was added to create a paste suitable for extrusion; the amount of water added to the silage diets before extrusion was decreased to take into account the water already added as part of the silage. The commercial hydrolysed protein was in powder form and no adjustment was made to the water added. All diets were prepared through extrusion at 70 °C, followed by drying in a ventilated oven at 55 °C. The final diets were presented as flat rectangular flakes with approximate dimensions of 20 mm × 30 mm. The commercial diet used in the control also served as the basal diet for all experimental diets. Proximate composition of the experimental feeds was determined according to standard AOAC methods (AOAC, 2003) and is shown in Table 2.

2.3. Experimental system and procedures

All feeding trials were conducted at Wild Coast Abalone Farm, Haga-Haga, South Africa, according to commercial culture conditions for South African abalone *H. midae*. Experimental animals were housed in plastic baskets under natural photoperiod, fed once daily to apparent satiation by experienced personnel, and uneaten feed was removed. The system had continuous supply of aeration and unfiltered seawater as extracted from the nearby ocean. Mean animal weight was determined at trial initiation and termination, as the mean weight of 30 randomly selected animals from each basket. Initial mean animal weight ranged between 123 g and 128 g, without any statistically significant differences between treatments (refer to Table 3). At the conclusion of the trial, one animal from each basket was also sampled for the determination of immune function.

Daily weight gain (DWG), feed conversion ratio (FCR), relative feed intake (RFI) and total mortalities were evaluated as indicators of production performance of each treatment. DWG was expressed as $mg \cdot animal^{-1} \cdot day^{-1}$ between sampling time i and $i + 1$ and was calculated as $DWG = 1000 \times (W_{i+1} - W_i) / (t_{i+1} - t_i)$ with W

Table 2
Diet formulation and proximate composition.

Ingredient ($g \cdot kg^{-1}$)	C	SL	SH	HPL	HPH
Abalone grower	975	920	833	968	950
Trout silage oil	25	24	15	25	20
Trout silage (wet)	0	56	152	0	0
HP1 (dry)	0	0	0	7	30
Total	1000	1000	1000	1000	1000
Proximate composition of experimental diets ($g \cdot kg^{-1}$)					
Moisture	86	89	79	77	71
Ash	79	79	80	84	82
Crude fat	58	62	58	63	57
Crude protein	389	392	405	395	404
Carbohydrates[a]	388	378	378	381	386
Gross energy ($MJ \cdot kg^{-1}$)	18.2	18.3	18.4	18.4	18.5

[a] Determined by difference.

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