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Fishmeal with different levels of biogenic amines in aquafeed: Comparison of feed protein quality, fish growth performance, and metabolism

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ABSTRACT

The current study investigated the effects of fishmeal quality (low (LB) and high (HB) levels of endogenous biogenic amines) and feed extrusion temperatures (100 and 130 °C) on protein oxidation indicators and amino acids racemization (AAR) in extruded fish feed. Furthermore, the study investigated the accompanying effects on feeding the diets to juvenile rainbow trout (*Oncorhynchus mykiss*) on fish growth performance, *in vivo* amino acids (AAs) digestibility, and liver and plasma metabolites following an 8-week feeding trial. A principal component analysis (PCA) showed that better growth performance, secondary oxidation products, and racemized methionine correlated positively with a low content of biogenic amines, whereas the primary oxidation product, protein hydroperoxides, and *in vivo* AAs digestibility correlated positively with high content of biogenic amines. At an extrusion temperature of 100 °C, the growth performance of the fish decreased when the content of biogenic amines increased. In contrast, at an extrusion temperature of 130 °C, the growth performance was unaffected by the level of biogenic amines. The latter could be a consequence of the higher level of protein oxidation of LB fishmeal compared to HB fishmeal at this temperature. Higher levels of liver pyruvate and plasma lactate together with high level of betaine and AAs in both liver and plasma were associated with the LB fishmeal diets. The lower concentration of AAs especially in liver of fish fed with HB fishmeal demonstrated that these AAs might not be supplied sufficiently for the tricarboxylic acid cycle to generate energy and therefore a decreased growth was found in fish fed this diet. Furthermore, the results indicated that biogenic amines and feed attractants such as betaine are more decisive for evaluating the quality of fishmeal than protein quality parameters.

1. Introduction

Factors such as the balance of essential amino acids, excellent palatability properties, and high protein digestibility have made fishmeal the prime protein source of the aquafeed industry. The quality of fishmeal depends on the industrial fish species, fishing method applied, type of raw materials included (e.g., whole fish, processing by-products or by-catch), raw material freshness, and fishmeal processing method, all of which may vary considerably between different suppliers and time of year (Tacon, 1993; Tacon and Metian, 2008).

Fish feed with fishmeal deriving from different sources and produced in different manners have been shown to affect protein digestibility as well as growth and feed utilization in fish (Caballero et al., 1999). For economic reasons it is therefore essential to find an appropriate way to evaluate the quality of fishmeal and avoid inconsistencies in feed optimization caused by such variations.

High quality fishmeal is considered to be low in biogenic amines and lead to a high protein digestibility (Aksnes and Mundheim, 1997; Mundheim et al., 2004). Hence, fishmeal produced from stale raw materials with a high content of biogenic amines has previously been shown to reduce the specific growth rate (SGR) of Atlantic salmon (*Salmo salar*) and survival, feed consumption and final biomass of blue shrimp (*Litopenaeus stylirostris*) compared to fishmeal produced from fresh raw materials (Opstvedt et al., 2000; Tapia-Salazar et al., 2004). The effects were related to a reduction in amino acids (AAs) availability due to bacterial degradation caused by unfavourable fishmeal storage

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conditions.

It has also been shown that the feed processing conditions affect the AAs digestibility and bioavailability in fish (Moksness et al., 1995; Aksnes et al., 1997). Furthermore, due to the dual role of the extrusion process on protein modifications, the final quality of proteins in extruded fish feed depends on the oxidative status of the proteins prior to extrusion. Hence, during the extrusion process moderately oxidized proteins may revert back to a more digestible form due to an increment in protein denaturation (Jung et al., 2014). On the other hand, unfolding of less oxidized proteins might expose different sites on the proteins to the oxidative environment inside the extruder and lead to a reduction in protein digestibility (Singh et al., 2007; Dunlop et al., 2009; Navale et al., 2015). Therefore, in addition to applying typical fishmeal quality judgement parameters, the influence of the extrusion process and the interactions of the feed ingredients with the protein quality after extrusion need to be taken into account.

Recent studies have shown that changes in dietary ingredients caused by production conditions of either fishmeal or extruded feed are reflected in fish tissues or biofluids (Wagner et al., 2014; Jasour et al., 2017). However, to our best knowledge, no study has focused on fish metabolites to understand why fishmeal with high content of endogenous biogenic amines affects growth performance. The aim of the present study was to combine feed protein quality parameters with growth performance data and associated changes in metabolites to achieve a better understanding of how fishmeal quality affects the fish metabolisms and growth performance. Consistent with this, we studied the effect of two fishmeals different in content of endogenous biogenic amines and fish feed extrusion temperature (100 and 130 °C) on protein oxidation indicators and amino acids racemization (AAR) in the extruded feed as well as subsequent effects on fish growth performance, AAs digestibility, and liver and plasma metabolites.

2. Materials and methods

2.1. Extrudates, feed, and fish experimental

Two isocaloric and isonitrogenous diets including two different fishmeals with either a low (LB) or high (HB) content of total biogenic amines were formulated (Tables 1 and 2), adding yttrium as an inert marker. The fishmeals were from different catches and had been processed differently by different suppliers. Therefore, they were different in total content of endogenous biogenic amines (Table 1). However, both fishmeals had comparable protein and lipid content and fulfilled the amino acids requirement of rainbow trout. Each of the two diets was extruded at two different temperatures (100 and 130 °C) using a twin-screw extruder (model BC 45, Clextral, France) mounted with a 2.4 mm die. The temperature range was chosen to maintain desired physical attributes such as density and durability with similar energy input. The screw speed, feeding rate, and specific mechanical energy (SME) were set as follows: 350 rpm, 140 kg/h, and 39–45 kW/h, respectively. The extruded diets were dried and coated with fish oil and rapeseed oil.

Table 1
Nutrient composition and level of biogenic amines for the two fishmeals used in the feed formulation.

		LB fishmeal	HB fishmeal
Proximate composition (g/100 g DM)	Protein	70.3	70.2
	Fat	11.7	9.2
	Moisture	6.13	6.8
Biogenic amines (mg/kg)	Putrescine	673	1620
	Cadaverine	1680	1830
	Histamine	108	873
	Spermidine	48.4	44.7
	Spermine	23.1	27.7
	Tyramine	711	760
	Tryptamine	27.8	111
	Phenethylamine	77.2	119
	Total BAs	3348.5	5385.4

LB: low biogenic amines, HB: high biogenic amines. DM: dry matter. The nutrient composition were measured as follows: crude protein (Kjeldahl nitrogen (N) ×6.25), crude fat (Soxtech apparatus), and moisture content (110 °C, 24 h).

Table 2
Formulations and nutrient composition for the meal mixes, extrudates, and feeds.

Formulations (g/100 g)[a]	LB fishmeal diet	HB fishmeal diet
LB fishmeal	53.1	
HB fishmeal		52.6
Wheat flour	18.0	19.7
L-Histidine	0.08	0.16
Yttrium	0.05	0.05
Fish oil	8.50	6.40
Rapeseed oil	17.7	19.1
Nutrient composition of the meal mixes[b]		
Protein (g/100 g DM)	53.2	51.2
Lipid (g/100 g DM)	7.9	9.1
Moisture (g/100 g)	8.6	9.5
Nutrient composition of the extrudates		
Protein (g/100 g DM)	56.0	53.3
Lipid (g/100 g DM)	8.4	9.5
Moisture (g/100 g)	6.8	6.9
Nutrient composition of the coated feeds		
Protein (g/100 g DM)	39.4	38.9
Lipid (g/100 g DM)	32.1	32.5
Ash (g/100 g)	8.4	7.07
Moisture (g/100 g)	7.7	7.9
Energy (MJ/kg)	24.5	24.4

LB: low biogenic amines, HB: high biogenic amines, DM: dry matter.

[a] The rest of ingredients (~2 g/100 g) was provided by mixture of minerals and vitamins.

[b] Mixture of ingredients sampled after meal mixer and before pre-conditioning. The nutrient composition were measured as follows: crude protein (Kjeldahl nitrogen (N) ×6.25), crude fat (Soxtech apparatus), dry matter (110 °C, 24 h), ash (550 °C, 6 h), and energy (Bomb Calorimeter). The nutrient composition of meal mixes, extrudates, and coated feed is based on dry matter unless otherwise indicated.

Samples labeled 'extrudate' were sampled immediately after the extrusion process at die and dried at room temperature. The dried extrudates were then stored in closed plastic containers at 4 °C until analysing for oxidation and heat-induced products. Samples named 'feed' refer to the extrudates after drying and oil coating and were stored in bags at 4 °C until used in the fish trials. The meal mixes, extrudates, and experimental diets were analysed for crude protein (Kjeldahl nitrogen (N) ×6.25), crude fat (Soxtech apparatus), dry matter (110 °C, 24 h), and energy (bomb calorimeter) (Table 2) using the EU approved methods "EU 152/2009" by Eurofins Steins Laboratorium A/S Denmark.

A feeding trial with juvenile rainbow trout (*Oncorhynchus mykiss*) was performed at the Biomar Research Center (Hirtshals, Denmark). The experiments were carried out in accordance with EU legislation and Danish Animal Welfare Regulations. A total of 1080 rainbow trout of initial weight of 111.5 ± 2.4 g were randomly allotted to 12 tanks (800 L, 90 fish per tank). After a 2-week adaptation period, triplicate tanks of fish were randomly assigned to one of the four experimental diets and fed four times a day to apparent satiation over the 8-week feeding trial. During the feeding trial, feed buckets were weighed to quantify consumption and special care was taken to ensure that fish had eaten all the feed. However, any uneaten pellets were collected and weighted in order to calculate the feed intake (FI). The water temperature was maintained at 12 °C, oxygen was above 92% throughout the study, and a light:dark ratio of 16:8 h was applied.

The daily specific growth rate in % day^{-1} (SGR, 100 × (ln final weight − ln initial weight) / days), feed conversion ratio (FCR, dry feed intake/wet weight gain), and daily feed intake in % day^{-1} (FI, 100 × total feed consumed/days) were calculated for each replicate at