



## Field monitoring of feed digestibility in Atlantic salmon farming using crude fiber as an inert marker



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### ABSTRACT

Modern feeds for industrial salmon farming are presently formulated to fulfill specific digestible protein (DP, grams per kg feed, hereafter called units) and digestible energy (DE, Mega Joule (MJ) per kg feed) specifications. In the present study, 299 feed samples and accompanying fecal samples were obtained from 43 different farming sites for Atlantic salmon along the coast of Norway between October 2010 and February 2013. Apparent digestibility coefficients for protein and fat were calculated from protein and fat in feed and fecal matter, using analyzed crude fiber contents as a digestibility marker. Overall mean apparent protein and fat digestibilities were 87.1% ( $\pm 4.9$ ; overall mean  $\pm$  S.D.) and 92.5% ( $\pm 3.7$ ), respectively. Linear mixed models with rearing site as random effect were applied to evaluate factors that influenced the apparent digestibility of protein and fat, feeding intensity and energy intake. Apparent digestibility of fat tended to increase over the study period, and both feed type and feed manufacturing company, and water temperature influenced the digestibility significantly. Digestible energy content (DE, MJ/kg) was significantly negatively associated with feeding intensity, but not with total digestible energy intake. Variation at site level was significant in all analyses, and was moderate for the digestibility analyses and high regarding feeding intensity and energy intake. Digestibility assessments were used to assess accuracy, precision and agreement between optimized and realized digestible protein (DP, %) and DE level of feeds, using the mean difference between the two sets of observations and a fixed contribution of dietary energy from starch. Overall mean difference between realized and optimized DP and DE was  $-0.28$  units and  $-0.56$  MJ/kg, respectively, and there were significant differences between feed manufacturing companies. The results indicated that field digestibility assessments can be used to validate nutritional and economical value of feeds in commercial salmon farming.

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### 1. Introduction

The rapid development of salmon farming has necessitated a parallel increase in fish feed production, and a concomitant search for protein and oil sources. Hence commercial feeds for carnivorous fish, that traditionally contained large amounts of meals and oils produced from pelagic marine fish, presently contain vegetable raw materials e.g. legumes and oil seed meals at the expense of marine ingredients (Drew et al., 2007a; Turchini et al., 2009). The cost and availability of plant proteins are superior to fish meal (FM), and this cost advantage may allow processing of crops to improve their nutritive value in finfish (Drew et al., 2007a). However, replacement of FM and fish oil (FO) with vegetable sources may present problems. Quality, concentration, and palatability of proteins from plant sources are generally inferior to marine sources (Drew et al., 2007a). Although carnivorous fish have a general preference for FO, most studies report that replacing FO with vegetable

lipid sources does not affect feed intake significantly (Turchini et al., 2009). This suggests that the lipids in the diet have little effect on palatability.

Measuring the digestibility of feeds and feed ingredients is a common and immediate way of assessing their nutritional value, and modification of dietary components can affect the digestibility of other nutrients as reviewed by Turchini et al. (2009). Plant protein ingredients have a lower and more variable nutritional value than FM, although digestibility appears to increase with increased processing (Drew et al., 2007a; Gaylord et al., 2009; Glencross et al., 2005). For lipids, the melting point can be considered as an indicator of the potential digestibility. Lipids with high content of saturated fatty acids lower fat digestibility, especially at lower water temperatures (Bendiksen et al., 2003; Ng et al., 2004; Turchini et al., 2009). Turchini et al. (2009) concluded that, in salmonids, replacement of FO with alternative lipid sources does not seem to have large effects on fish performance although long-term effects require further elucidation. Experimental trials have reported that water temperature and feeding level can affect nutrient digestibility (Azevedo et al., 1998; Bøgevik et al., 2010; Ng et al., 2004). However, nutrient and energy

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digestibility have not been investigated in commercial salmon production where the fish are reared at ambient environmental conditions throughout a whole production cycle.

Modern aquaculture diets are routinely formulated based on the digestible nutrient and energy criteria (Cho and Kaushik, 1990). Measurement of digestible energy (DE), digestible protein (DP), and digestibility of ingredients means measuring that amount of the energy or nutrient that is not excreted in feces. Two key methodological approaches are in use; the direct and indirect assessment methods (Glencross et al., 2007; Maynard and Loosli, 1969). In the direct method, complete accounts of feed inputs and fecal outputs are required, and the digestible value of the feeds is then determined on a mass-balance basis. Collection of accurate data on feed intake and fecal production is, however, a source of error even at laboratory conditions. By the use of the indirect method, representative samples of both the feed and the feces are required, and the ratio of an added indigestible marker in the feed and feces determines dry matter digestibility. This is used to calculate apparent digestibility of fat and other nutrients (Glencross et al., 2007). Different marker types have been used in aquaculture nutrition digestibility studies: chromic oxide, rare earth metal oxides such as ytterbium oxide and yttrium oxide, hydrocarbon markers such as cholestane, and endogenous markers such as acid-insoluble ash and crude fiber (Austreng, 1978; Austreng et al., 2000; Carter et al., 2003; Morales et al., 1999; Ringø, 1993). Acid-insoluble ash has been reported unsuitable as a marker, while crude fiber has been shown to be an effective marker, although the source of vegetable feedstuff in the feed might influence the results (Morales et al., 1999). Most studies of digestibility assessments have been performed in experimental settings with standardized environments and over time periods of shorter duration. Thus the value of such assessments under commercial rearing conditions can be questioned. Therefore measuring nutritional value by digestibility assessments on site based on a raw material borne component could be useful as a tool for monitoring and validation of feeds and feed performances in commercial salmon production, and also serves as a quick validation tool for new feeds and feed raw materials.

The aims of this study were to (1) evaluate the feasibility of assessing nutritional value from nutrient and energy digestibility, using crude fiber as an inert marker to (2) identify main factors which affect digestibility of protein and fat, feeding intensity (%), and total digestible energy intake, and (3) describe accuracy and precision in monitoring DE and DP measured as the difference between optimized and realized DE and DP by use of field data from commercial salmon production.

## 2. Materials and methods

### 2.1. Study design and data material

Feed samples and accompanying fecal samples of farmed Atlantic salmon from one large Atlantic salmon farming company in Norway were obtained during commercial production between October 2010 and February 2013. The material included 299 digestibility assessments obtained from 207 cages at 43 different rearing sites along the coast of Norway (between 62.0° and 70.6°N). The sampled fish were > 1 kg live weight and were held in circular cages of 120 or 157 m in circumference. The fish were fed commercial extruded pelleted feeds from five feed manufacturing companies. The feed products were fed the fish to apparent satiation on a daily basis including day of fecal collection. A central feed system were used where feed were blown from feed silos on the barge to the nearby rearing cages through floating polystyrene pipes using controlled high pressure air. Each pipe was equipped with a rotating spreader at the end to provide optimal feed spread in each cage.

### 2.2. Fecal sample collection

At sampling, the fish were collected from the production cage using a sweep net. The fish were netted at random and anesthetized in

benzocaine (Benzoak® vet. 200 mg/ml, ACD Pharmaceuticals AS). Feces were collected by the stripping method (Austreng, 1978), until a pooled sample of approximately 70 g wet weight had been collected from each cage. One to three cages per site were sampled monthly for fish > 1 kg.

### 2.3. Feed and fecal sample analyses

Feed samples were taken concurrently with fecal sampling to ensure representative samples for digestibility assessment. The feed and fecal samples were stored frozen at  $-40\text{ }^{\circ}\text{C}$  for 1–7 days before being sent to an external accredited laboratory (Nofima AS, Bergen, Norway). Fecal matter was freeze dried before feed and feces samples were analyzed for protein, fat and crude fiber using accredited methods. Feed and fecal samples were analyzed for dry matter ( $105\text{ }^{\circ}\text{C}$  for 24 h) and crude protein ( $N \times 6.25$ , Kjeltac Autoanalyser, Tecator, Sweden). Crude fat was estimated on acid hydrolyzed samples (3 M HCl) using the Soxhlet method with petroleum ether extraction. Analyses of crude fiber (AOAC 978.10) (AOAC, 2000) were also performed (crude fiber comprised 0.5–4% of the feeds). Nutrient digestibility was assessed by the indirect method using crude fiber as the inert marker (Glencross et al., 2007). Apparent digestibility coefficients (%) of nutrients (N), being fat and protein were calculated as follows:

$$\text{ADC \%} = 100 - 100 * \left[ \left( \frac{M_{\text{feed}}}{M_{\text{feces}}} \right) * \left( \frac{N_{\text{feces}}}{N_{\text{feed}}} \right) \right]$$

where  $M$  is the marker (crude fiber) and  $N$  is either crude protein (P) or crude fat (F) in feed and feces. Dietary digestible protein content (DP, %) was calculated from dietary protein content and the apparent digestibility coefficient of protein, and the realized DE (MJ/kg) in feed was estimated as the sum of digestible energy from fat, protein and starch (S):

$$\text{DE} = (F_{\text{feed}} * \text{ADC}_{\text{fat}} * 39,5 \text{ MJ/kg}) + (P_{\text{feed}} * \text{ADC}_{\text{protein}} * 23,6 \text{ MJ/kg}) + (S_{\text{feed}} * 65\% * 17,4 \text{ MJ/kg}).$$

Energy contribution from starch was estimated by setting starch at a fixed value of 8 g per 100 g of feed at a digestibility of 65%. Gross energy in feed was set to 39.5 MJ/kg for fat, 23.6 MJ/kg for protein and 17.4 MJ/kg for starch (Blaxter, 1989).

### 2.4. Explanatory variables – registrations

Factors considered for potential influence on digestibility of protein and fat were site production area (a measure of latitude), water temperature in degree centigrade ( $^{\circ}\text{C}$ ) at 5 m depth, feed company, feed type, optimized DE and DP in the feed, feeding intensity (percentage of biomass/day), average fish size (grams), age of smolt (s0, s1, s1.5), and genetic strain. In addition, feed given on day of sampling, biomass (tons), and number of fish per cage were recovered from the production control system. There were different types of feeds with different nutrient contents in use. The different feed types were grouped according to nutrient content and fish size due to the fact that there are greater differences in performance of the high performance feed for large fish compared to high performance feed for small fish. According to this, feed type was classified as standard feeds, high performance (nutrient dense) feeds, or other types of feeds. Although there were minor differences between the feed manufacturing companies the standard and high performance feeds were further subdivided into feed for fish of live weight 1–2 kg ('1000 products') and feed for >2 kg fish ('2000 products').

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