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Addition of yttrium oxide as digestibility marker by vacuum coating on finished pellets — A method for assessing digestibility in commercial salmon feeds?

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ABSTRACT

Two experiments were carried out with Atlantic salmon in sea cages to study the possibility of assessing digestibility of commercial feeds by means of an inert marker added by top coating in a vacuum coater. A complete salmon feed (39% protein, 34% lipids) containing ytterbium oxide (Yb_2O_3) added pre-extrusion was top-coated with yttrium oxide (Y_2O_3) dispersed in fish oil. The feed was fed to salmon (2–3 kg) in two experiments. Digestibility estimates using the two methods of marker addition were compared in stripped faeces in Experiment 1, and in Experiment 2 temporal variations in marker-to-marker and nutrient-to-marker ratios in the content of different gastrointestinal segments after a single meal were studied.

Digestibility measured using the two markers showed consistent results from stripped fish. However, data from Experiment 2 showed that yttrium (Y) dispersed in oil and added by top-coating was evacuated from the stomach together with lipids a short time after ingestion, while evacuation of protein and ytterbium (Yb) added to the meal mix pre-extrusion followed later. The Y:Yb ratio in the mid-intestine remained higher than in the feed until 17 h after feeding and until 24 h in the distal intestine.

In conclusion, yttrium oxide added by top-coating can be used as a marker for digestibility assessment in commercial salmon feeds. Measurements may possibly be biased, however, by the differences in gastrointestinal evacuation rates amongst nutrients and markers, so frequent feeding and/or careful timing of the faeces collection may be required for a reliable result.

1. Introduction

Digestibility, particularly of protein and energy, is an important quality criterion for fish feeds. It is most commonly measured in experimental systems by the indirect method, using an inert marker added to the feed (Maynard and Loosli, 1969). Faeces may be collected by different methods, including careful stripping of the fish (Austreng, 1978), dissection of the intestine or different ways of collection from the water outlet (Cho and Slinger, 1979; Choubert et al., 1979, 1982; Glencross et al., 2007). Stripping is the preferred choice for salmon in sea cages since collection of faeces from the water is not practically feasible (Percival et al., 2001). This method is also less sensitive to the physical structure and water stability of the faeces, factors known to be affected by dietary raw material composition, e.g. by inclusion of soya products (Olli and Krogdahl, 1994). It may, on the other hand, be more sensitive to temporal variations in faecal marker-to-nutrient ratios than continuous faeces collection (Hajen et al., 1993; Ward et al., 2005).

Different markers have been used, but yttrium oxide (Y_2O_3) has become dominant in studies with Atlantic salmon (Austreng et al., 2000). Since markers used in experimental systems are either too expensive or have other properties excluding the possibility of adding them in commercial feed production systems, it has been difficult to evaluate the digestibility of commercial feeds. A thoroughly validated method based on natural components in commercial diets has not yet been established. Crude fibre has been used by some authors (Bendiksen et al., 2011; Krontveit et al., 2014), but this method lacks validation. Morales et al. (1999) tested acid insoluble ash (AIA) and crude fibre as possible markers, in comparison to chromium oxide (Cr_2O_3), in rainbow trout. Even after addition of celite and cellulose to obtain levels of AIA and fibre far above present levels in commercial feeds, the digestibility coefficients obtained with AIA and fibre were not consistent with those obtained with chromium oxide.

Attempts have been made to add digestibility markers to commercial feed. Hillestad et al. (1999) coated yttrium oxide with a small amount of oil in a concrete mixer, but concluded that the loss of yttrium (Y) to the

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water was too high. The method recommended was grinding the dry feed and mixing in the marker before making a moist diet (Hillestad et al., 1999). The obvious disadvantage with this method is that the water content and physical structure of the feed are markedly changed after re-processing and effects on digestibility cannot be excluded.

The objective of the present study was to investigate if nutrient digestibility of commercial diets can be determined by top-coating the diets with an inert marker using a vacuum coater. A complete salmon feed was made with inclusion of a digestibility marker, ytterbium oxide (Yb_2O_3), homogeneously mixed in the dry feed mix before extrusion and vacuum coating with oil. The finished feed was then re-allocated to the vacuum coater and yttrium oxide was added, dispersed in a small amount of oil. Digestibility determined by top-coating of an inert marker could then be compared with digestibility determined by addition of marker the traditional way.

2. Materials and methods

2.1. Feed and feed production

The experimental feed was produced by extrusion at the feed technology centre of Nofima, Bergen, Norway. The basal diet was based on a high quality LT herring meal as the major protein source, wheat as binder, a 50:50 mix of fish oil and rapeseed oil as the major lipid source added by vacuum coating, and 100 mg/kg ytterbium oxide (Yb_2O_3) mixed with the dry ingredients prior to extrusion as an inert digestibility marker.

The basal diet was then added 250 mg/kg yttrium oxide (Y_2O_3) by top-coating. This was obtained by making a stock solution with 16.7 mg Y_2O_3 per g fish oil. The yttrium oxide was added to pre-heated oil (45 °C) followed by rigorous mixing with a vortex blender in combination with sonication for several minutes until the yttrium oxide was well dispersed. Then 150 g of the stock solution was added to 10 kg of the basal diet under vacuum in a vacuum- and top coater (Dinnissen, Sevenum, Holland). The oil and feed were mixed before air was released back into the coater. Six coated batches of 10 kg were then mixed and two random samples of the feed were taken for analyses of digestibility markers and nutrient composition as described below. The composition of the finished feed is given in Table 1. Eight additional samples were taken randomly and analysed for Y in order to check homogeneity, showing a CV of 4.0%.

A test was done to study the loss of coated Y from pellets in seawater. Weighed pellet samples (50 g) were added to 1 l of seawater (11 °C) and gently stirred for 0.5, 1.0, 2.0 or 4.0 min. The seawater was then removed and the sample washed with 1 l of seawater in a colander. The retained feed was weighed and analysed for dry matter, Y and Yb, and retention of markers calculated as % of the amount in the initial feed sample.

2.2. Experiment 1: digestibility

A feeding trial was carried out in a sea cage at the research station of Nofima, Averøy, Norway (63°, 03′ N, 7°, 35′ E). The cage (5 × 5 × 5 m) was stocked with 100 Atlantic salmon (approx. 3 kg individual weight) and fed the experimental diet in three 30 min meals per day by automatic feeders (Betten Maskinstasjon AS, Vågland, Norway). The feeding periods were 09.00–09.30 h, 12.10–12.40 h and 14.35–15.05 h. Faeces for determination of nutrient digestibility were collected after 22 days of feeding, after the morning meal (10.00–12.00 h). Forty fish were dip-netted, anaesthetised in metacaine (MS 222), and gently stripped for faeces as described by Austreng (1978) to make two pooled faeces samples (20 fish per sample). The water temperature decreased from 8 to 6 °C during the experiment and was 6.4 °C at the day of stripping. The collected faeces were frozen, freeze dried and analysed for dry matter, nitrogen, lipids, amino acids, starch, indigestible markers, P and Zn as described below.

Table 1
Formulation of the basal diet and chemical composition of the experimental diet after top-coating.

Formulation of basal diet, g kg^{-1}	
Fish meal[a]	549
Fish oil[b]	133
Rapeseed oil	132
Wheat	150
Vitamin premix[c]	20
Mineral premix[d]	5
Monosodium phosphate	10
Carophyll Pink™, 10% astaxanthin	0.5
Ytterbium oxide (Yb_2O_3)	0.1
Chemical analyses	
Yttrium oxide, mg kg^{-1} (added in vacuum coater)	216
Ytterbium oxide, mg kg^{-1} (added in meal mash)	105
Crude fat, g kg^{-1}	343
Crude protein (N×6.25), g kg^{-1}	394
Ash, g kg^{-1}	72.6
Starch	99
Dry matter, g kg^{-1}	937
Phosphorus, g kg^{-1}	14.3
Zink, mg kg^{-1}	177
Essential amino acids, g kg^{-1}	
Arginine	26.3
Histidine	07.2
Isoleucine	15.8
Leucine	27.2
Lysine	26.3
Methionine	09.7
Phenylalanine	12.7
Threonine	14.8
Tryptophan	02.7
Valine	19.1
Non-essential amino acids, g kg^{-1}	
Alanine	22.5
Aspartic acid	30.0
Cysteine	3.3
Glycine	21.9
Glutamic acid	51.5
Proline	17.4
Serine	14.9
Tyrosine	9.9

[a] Herring meal, Egersund Sildeoljefabrikk AS, Egersund, Norway.
[b] NorSalmOil, Egersund Sildeoljefabrikk AS, Egersund, Norway.
[c] Provided per kg of feed: vitamin D3, 3000 I.E., 160 mg; vitamin E (Rovimix, 50%), 160 mg; thiamin, 20 mg; riboflavin, 30 mg; pyridoxine-HCl, 30 mg; vitamin C (Rovimix Stay C, 35%), 200 mg; calcium pantothenate, 60 mg; biotin, 1 mg; folic acid, 10 mg; niacin, 200 mg; vitamin B12, 0.05 mg; menadione bisulphite, 20 mg.
[d] Provided per kg of feed: magnesium 750 mg (as $MgHPO_4 + H_2O$); potassium, 800 mg (as K_2CO_3); zinc, 120 mg (as $ZnSO_4 + H_2O$); iron, 60 mg (as $FeSO_4 + H_2O$); manganese, 30 mg (as $MnSO_4 + H_2O$); copper, 6 mg (as $CuSO_4 + 5H_2O$); selenium 0.3 mg.

2.3. Experiment 2: gut transfer rate

A second experiment was carried out at the same site in order to study the evacuation rates of the markers through the gastrointestinal tract. A cage (5 × 5 × 5 m) was stocked with Atlantic salmon (approx. 2.4 kg) and fed a commercial diet (9 mm pellets, Skretting AS, Averøy, Norway) for one week to ensure that the fish had normal appetite. The fish were then fasted for two days, and then hand fed ad libitum in one meal lasting for 30 min. The water temperature was 10.5 °C.

The content of the stomach, the mid-intestine (including pyloric caeca) and the distal intestine was sampled from 5 fish at 3, 6, 11, 17, 24 and 36 h post-feeding. The fish were killed by an overdose of metacaine and dissected. The gastrointestinal (GI) tract was closed by artery clamps at the oesophagus and anus. The passages between the stomach and the gut, and between the mid-intestine and the distal intestine, were closed by plastic strips. The whole GI tract was then dislocated and frozen at −25 °C to ease the removal of the gastrointestinal content. The contents of the different segments were freeze dried