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Analytical Methods

A fast and simple methodology for determination of yttrium as an inert marker in digestibility studies

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Abstract

The need to develop a fast, simple and low cost methodology for the determination of yttrium in fish diets and faeces using a microwave digestion system and atomic absorption spectrometry with flame atomization analysis was the main aim of this study. The final methodology consisted in the digestion of approximately 300 mg of dry fish diet or 100 mg of dry fish faeces in teflon vessels using Parr reactor bombs, at high pressure, in a domestic microwave system with nitric acid solution. After digestion, 330 μl of a 120 mg potassium nitrate/ml solution was added to each solution. Yttrium was determined using a calibration with aqueous standards. Analytical difficulties and problems encountered during the optimizations were overcome and the application of the final methodology to the fish diets and faeces samples was carried out with suitable results.

Keywords: Microwave digestion; Atomic absorption spectrometry; Yttrium; Inert marker; Fish diets; Fish faeces; Digestibility studies

1. Introduction

The digestibility of proteins, lipids, energy components and individual amino acids are normally estimated by the apparent digestibility coefficients (ADC) and should be in consideration as the basis for feed formulation in fish (Austreng, Storebakken, Thomassen, Refstie, & Thomassen, 2000; Davies & Gouveia, 2006; Garatun-Tjeldsto, Ottera, Julshamn, & Austreng, 2006; Hung, Berge, & Storebakken, 1997; Refstie, Helland, & Storebakken, 1997; Regost, Arzel, Robin, Rosenlund, & Kaushik, 2003; Sugiura, Dong, & Hardy, 1998; Ward, Carter, & Townsend, 2005).

A standard approach in digestibility studies is the incorporation of an inert maker compound into the fish diets, normally chromium oxide (Cr_2O_3). Nevertheless, this marker is not totally recovered in faeces and its dietary incorporation level has to be quite high (0.5–1%) in order to obtain homogeneous analytical results (Lied, Julshamn, & Braekkan, 1982). High dietary levels of chromium oxide may affect absorption and metabolism of nutrients in fish (Ringø, 1993a; Ringø, 1993b; Ringø, 1993c). Recent studies have suggested the suitability of yttrium oxide as an inert digestibility marker as should not affect the fish metabolism and can be included at low concentrations (in the mg kg^{-1} range) (Austreng et al., 2000; Davies & Gouveia, 2006).

An optimized analytical method for the determination of yttrium oxide is essential in this type of studies. The decomposition of the sample is a critical step as it can have important effects on the analytical results (Blust, Van der Linden, Verheyen, & Decleir, 1988). One must assure total decomposition of the sample and also that, during decomposition, no losses of the elements that are to be determined occurs. Several methodologies can

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be found for the pre-treatment of the fish diets and faeces samples. The most common pre-treatment is the combustion of these samples at high temperature and digestions with acid solution (Austreng et al., 2000; Bjerkeng et al., 1997; Hillestad, Asgard, & Berge, 1999; Hung et al., 1997; Refstie, Storebakken, & Roem, 1998; Refstie et al., 1997; Storebakken, Kvien, Shearer, Grisdale-Helland, & Helland, 1999; Storebakken, Shearer, Refstie, Lagocki, & McCool, 1998; Sugiura et al., 1998; Thodesen et al., 2001; Ward et al., 2005; Xue & Cui, 2001; Xue, M, Xie, & Cui, 2004; Ytrestoyl, Struksnaes, Koppe, & Bjerkeng, 2005), normally, a time consuming procedure. But, recently, a microwave digestion procedure has been used (Davies & Gouveia, 2006; Garatun-Tjeldsto et al., 2006), a much faster procedure. The microwave digestion devices still imply a considerable investment in any laboratory, because they are invariably costly. A cheaper option is the use of Parr reactor bombs in combination with conventional domestic microwave ovens, which to our knowledge has never been used before for the decomposition of fish diets and faeces.

Normally, yttrium analysis is carried out by inductively coupled plasma spectrometry (Austreng et al., 2000; Bjerkeng et al., 1997; Davies & Gouveia, 2006; Garatun-Tjeldsto et al., 2006; Hillestad et al., 1999; Hung et al., 1997; Refstie et al., 1997; Refstie et al., 1998; Storebakken et al., 1998; Storebakken et al., 1999; Sugiura et al., 1998; Thodesen et al., 2001; Ward et al., 2005; Xue & Cui, 2001; Xue et al., 2004; Ytrestoyl et al., 2005), but atomic absorption spectrometry (AAS) can also be used (Regost et al., 2003) as long as the instrumental parameters for yttrium determination are carefully optimized.

The aim of this work was the development of a fast, simple and low cost methodology for the determination of yttrium in fish diets and faeces using microwave digestion in Parr reactors bombs and atomic absorption spectrometry analysis.

2. Experimental

2.1. Materials

High-purity bideionised water from a Milli-Q system (conductivity: 0.054 μS/cm at 25 °C) and concentrated nitric acid (Fluka, analytical grade) were used throughout the work. The concentrated nitric acid was used without further purification and all the others reagents used in this work were of analytical or equivalent grade. The standard yttrium solutions used in the analyses were daily prepared from stock solutions of 1000 mg/L, in polyethylene tubes, by weight.

To prevent contamination, all materials used in the sampling and in the treatment of samples were soaked in a 20% (v/v) nitric acid solution at least for 24 h, washed several times with deionised water and dried in an oven.

2.2. Samples preparation

Four experimental diets were formulated to contain two different protein (P)/lipid (L) ratio levels (60P/6L or 50P/10L) and two different protein sources (either fish meal (FM) or 50% FM and 50% plant meal (PM)). To a grounded portion of each of the four diets, 0.1% of yttrium oxide, as inert marker, was incorporated. The mixtures were then dry pelleted through a 2.4 mm die at 50 °C (CPM, C-300 model). Eight homogenous groups of fish (*Pagellus bogaraveo*) were stocked into a specially constructed digestibility system according to the Guelph system protocol (Cho, Slinger, & Bayley, 1982). Fish were fed once daily until apparent satiety with each experimental diet (each diet was allocated to two tanks) and faeces from each tank were collected every day over a four week period. After collection, faeces were centrifuged and frozen at –20 °C. Faeces were subsequently freeze-dried before further analysis. The fish diets and faeces samples were then homogenised in a mortar.

For yttrium analysis, in the final methodology, approximately 300 mg of dry diet or 100 mg of dry faeces were digested in teflon vessels using Parr reactor bombs (model no. 4782, Parr), at high pressure, in a domestic microwave system (model no. NE-1037, Panasonic). The digestion program consisted in the addition of a solution of 2.75 ml of bideionised water with 0.75 ml of concentrated nitric acid to the samples, a microwave digestion at 340 W for 4 min, a cooling stage of at least 2 h in an ice bath followed by the addition of 0.25 ml of concentrated nitric acid and a second microwave digestion at 340 W for 4 min. Before dilution to 10 ml (diets) or 15 ml (faeces) with bideionised water, 330 μl of a 120 mg potassium nitrate/ml solution was added to each digested solution.

2.3. Instrumentation

Yttrium concentration in the digested solutions was determined with an atomic absorption spectrometer (SpectrAA 220 FS, Varian) with a deuterium lamp for background correction and with flame atomization (Marck 7, Varian). For yttrium determination it was used a nitrous oxide – acetylene flame (9.76–6.89 L/min) using an absorbance of 420.2 nm and a slit width of 0.5 nm.

Comparisons between external calibrations with aqueous standards and calibrations by standard addition were used to check the suitability of the digestion and of the analytical procedure in the preparation and analysis of fish diets and faeces samples.

The need for the addition of potassium nitrate to both standards and samples was also checked.

Blank solutions were prepared following the pre-treatment of the samples. Three independent replicates of each sample were prepared and analyzed and, after blank subtraction, mean values and respective standard deviations were calculated.

Statistically significant differences among samples for 5% level of significance were evaluated through ANOVA tests using SPSS software and Tukey pairwise comparisons were used.

3. Results and discussion

3.1. Optimization of the analytical procedure for fish diets samples

3.1.1. Evaluation of the suitability of the samples digestion for the determination of yttrium

The procedure for the pre-treatment of the sample was adapted from that optimized for biological tissues (Reis & Almeida, 2008). In a first stage, to evaluate the suitability of the digestion, a known amount of solid yttrium oxide was digested, using the microwave digestion procedure described in Section 2, and the yttrium recuperation was determined using a calibration with aqueous standards. An yttrium recuperation of 109% was observed which indicated that apparently there were no losses of yttrium during the digestion procedure.

To confirm the suitability of the pre-treatment, the four dry fish diet samples were spiked with two different amounts of yttrium before the digestion. The yttrium recuperations averaged 121 ± 9%. Thus, there seems to be no losses of yttrium during the digestion and this pre-treatment can be used for the determination of yttrium in fish diets samples. Nevertheless, recuperations higher than 100% indicated that matrix effects were probably present in the analytical measurements and that the quantification procedure needed to be optimized.

3.1.2. Evaluation of the matrix effects and selection of the best method for calculation of yttrium

To evaluate matrix effects in the analytical measurements, two calculation methods were compared: calibration with aqueous standards and calibration by standard addition.

For standard addition calibrations, a digested fish diet solution was spiked with different amounts of yttrium and analysed. As observed in Fig. 1, the linear adjustment of each calibration, standard addition or aqueous standards, had significantly different slopes indicating that matrix effects were in fact present.

Using the atomic absorption spectrometry (AAS) with flame atomization, yttrium can be partially ionized in the nitrous oxide–acetylene flame (Varian, 1989). This ionization will probably be dependent of the matrix of each solution analysed and can be the cause of the observed matrix effects. To suppress ionization, potassium should be added to all solutions (Varian, 1989), both samples and standards. Therefore, potassium nitrate solution was added to each standard and to each sample solution and the two types of calibrations were compared once again.

As observed in Fig. 1, the slopes of both calibrations were statistically identical indicating that matrix effects were overcome. These results suggested that yttrium determination in fish diet samples can be carried out by aqueous standards calibration, as long as, potassium nitrate is added.

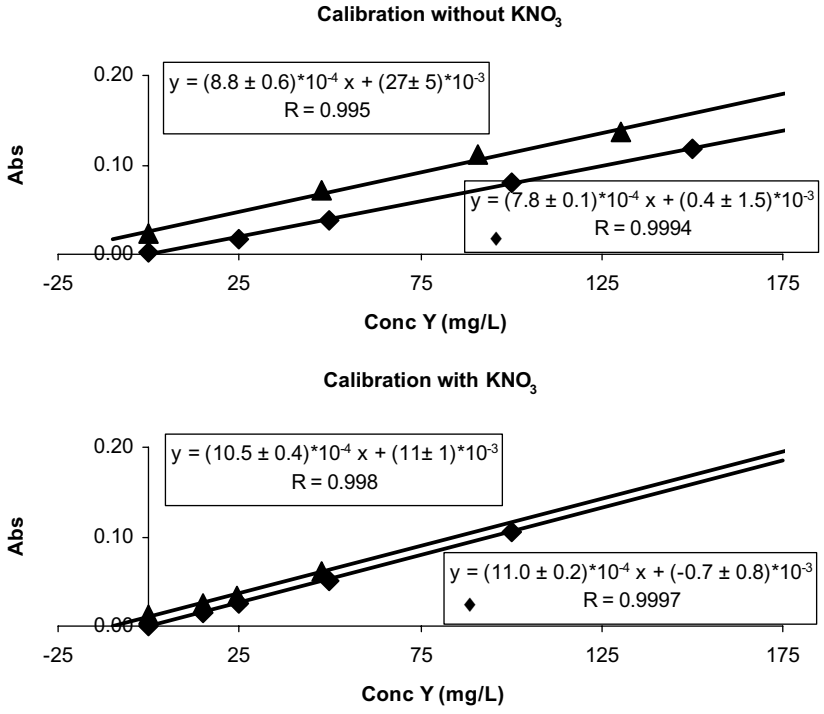

Fig. 1. Evaluation of the matrix effects with and without KNO_3, calibration either through aqueous standards (♦) or through standard addition (▲).

3.1.3. Application of the analytical method to the fish diet samples

To test the suitability of the analytical methodology, the four different fish diets samples that were mixed with 0.1% yttrium oxide (see Section 2), were again digested in the microwave system using the selected pre-treatment and potassium nitrate solution was added to all digested solutions.

The determination of yttrium was made using the aqueous standards calibration and analysing directly the digested diet samples without dilutions.

As observed in Table 1, the percentage of recuperation of yttrium in the spiked samples averaged 87 ± 18%, which validates the selected analytical methodology. Moreover, the yttrium recuperation did not differ significantly among diets indicating that the pre-treatment was independent of the different levels of lipids and proteins in the diets.

The detection limit of the entire procedure was 0.170 mg Y/g of dry fish diet.

3.2. Optimization of the analytical procedure for faeces

3.2.1. Evaluation of the matrix effects and minimal mass of fish faeces sample

In a first stage, four fish faeces samples collected from the fishes fed with the diets containing 0.1% yttrium oxide (one duplicate per diet, see Section 2) were digested following the pre-treatment used for fish diet samples.

Preliminary tests showed that faeces digested solutions clogged the nebulisation system of the AAS equipment. Therefore, a higher dilution of the digested solution was required and after microwave digestion, faeces solutions were diluted to 15 ml instead of the 10 ml selected for fish diet solutions (see Section 2).

Digested faeces solution was then spiked with known amounts of yttrium and determination of yttrium was performed using the aqueous standards calibration used for digested fish diets solution.

The percentages of recuperation of yttrium averaged 87 ± 9% indicating that the analytical methodology selected for diet samples was also suitable for faeces samples.

Fish faeces are not easy to obtain and it is difficult to obtain a high amount of these samples. Therefore, it is important to reduce the mass of sample required for yttrium analysis. Hence, in a second stage, a lower amount of fish faeces was selected: 100 mg instead of 300 mg.

Therefore, for the four faeces samples the results obtained with the two digested masses were compared. Faeces samples showed 3.2 ± 0.1 mg Y/g dry sample and 3.6 ± 0.4 mg Y/g dry sample for 300 mg and 100 mg of digested mass sample, respectively. Thus, statistical identical results were obtained and the fish faeces mass sample to be digested can be minimized.

The digested solution of the lower mass was also spiked with known amounts of yttrium and an yttrium average recuperation of 105 ± 17% was obtained through calibration with aqueous standards.

3.2.2. Application of the optimized analytical method to the faeces samples

To test the suitability of the selected analytical methodology in digestibility studies, duplicates faeces samples collected from fish fed with each of the four experimental diets were analyzed after digestion.

The determination of yttrium was made by using the aqueous standards calibration. The concentrations of yttrium oxide in the faeces samples are shown in Table 1.

In the fish faeces, an amount of yttrium four times higher than that added to the fish diet is normally obtained. Therefore, in the present study a value of 4 mg Y_2O_3/ dry faeces was expected. As observed in Table 1, all values were closed to the expected value independently of the dietary treatment, and replicates of each sample were very good (variation coefficient less than 0.1%). Moreover, the yttrium recuperation (mg/g dry faeces) did not differ significantly among dietary treatments.

The detection limit of the procedure was 0.75 mg Y/g of dry faeces, which was slightly higher than that observed for fish diets due to the higher dilution required for faeces digested samples.

Table 1
Concentrations[a] of yttrium oxide in four experimental diets formulated to contain two different protein (P)/lipid (L) ratio levels (60P/6L or 50P/10L) and two different protein sources (either fish meal (FM) or 50% FM and 50% plant meal (PM))and in the faeces of fish fed with these diets (in duplicate for each diet)

Diet	Yttrium oxide (mg/g dry diet)	Yttrium oxide (mg/g dry faeces)
1 (60:6 FM)	0.8 ± 0.1	3.0 ± 0.1, 4.3 ± 0.2
2 (60:6 PM)	0.7 ± 0.1	4.4 ± 0.5, 4.4 ± 0.4
3 (50/10 FM)	1.1 ± 0.3	3.4 ± 0.4, 5.0 ± 0.3
4 (50/10 PM)	0.9 ± 0.1	3.4 ± 0.2, 4.5 ± 0.2

[a] Mean and standard deviation (n=3).

4. Conclusions

This study presents an optimized analytical method for the direct determination of yttrium in diets and faeces of fish through digestion in a microwave system with Parr reactor bombs and analysis by AAS with flame atomization.

Analytical difficulties and problems encountered during the optimizations and the application of the final method to the diets and faeces samples was carried out with suitable results.

The selected analytical methodology is simple, non expensive and allows a fast yttrium determination in food nutrition studies.

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