

Digestibility appraisal of commercially refined field pea and cereal derived protein concentrate products for use in practical diets for rainbow trout, *Oncorhynchus mykiss*

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Abstract

A digestibility trial was performed on production sized rainbow trout (110g mean weight) in order to test the efficacy of selected protein concentrate sources (pea protein, wheat and maize gluteins) as a partial replacement for fishmeal in balanced diets. These products substituted for 50% of the crude protein as fishmeal in a reference diet and yttrium oxide was included as the dietary marker for digestibility assessment. After adjustment to experimental diets, the digestibility of protein, essential amino acids, energy, lipid and carbohydrate was determined for rainbow trout under controlled conditions. All products yielded high crude protein and EAA digestibility coefficients with pea and wheat protein being highest at 95%. A lower value of 80% was obtained for the maize protein product. Amino acid availability reflected the protein coefficients and was over 95% in most cases. The lipid digestibility was marginally depressed for diets containing cereal proteins compared to the fishmeal reference diet. Carbohydrate digestibility was also reduced in these sources compared to the fishmeal fed group and consequently energy digestibility was similarly decreased. There were no negative effects on phosphorus availability with coefficients ranging between 50-58%. The findings are discussed in the context of applied fish nutrition and feed formulation for salmonid fish and rainbow trout in particular.

Key words

Pea, maize, wheat gluten proteins, protein & amino acid digestibility, rainbow trout

Introduction

Renewed focus on the partial or even the complete replacement of fishmeal with novel proteins is now of paramount interest due to public perception and demand for sustainable development and expansion of aquaculture (Hardy and Kissil, 1996). Although animal proteins have been shown to be of high biological value (BV) and offer a feasible alternative, there are still many restrictions in force due to the concerns of prion related TSE's and associated hygiene issues. This is particularly the case in Europe.

Alternately, plant proteins have been extensively forwarded as the main basis for the effective replacement of fishmeal and soy bean meals have been the major by-products with any considerable potential and merit. Their use has been advocated by many workers (Akiyama 1991; Oliva-Teles et al. 1994; Olli et al. 1995; Nengas et al. 1996) for many species of cultured fish across the spectrum of warm water, temperate, fresh water and marine species.

There have been considerable technical advances in the soy processing industry that has resulted in high quality materials with low anti-nutritional factors (ANF's) making them more suitable for inclusion in fish feeds.

Some of these by-products have the disadvantages of low or medium levels of protein, inferior amino acid balance and reduced digestibility due to the presence of ANF, undigested complex poly and oligosaccharides. Removal of the carbohydrate components in cereals and pulses enables the protein to be concentrated or isolated.

Thus, corn gluten meal is especially useful and is relatively free of these factors. Pereira and Pereira, T.G & Oliva-Teles (2003) evaluated corn gluten meal as a source of protein in diets for juvenile gilthead sea bream, *Sparus aurata*. They found that corn gluten could replace up to 60% of the fish meal protein with no negative effects on fish performance as measured by growth rate, feed utilisation and digestibility.

Other plant proteins include oilseed rape meal (canola) and a various pulses, legumes from different sources that have undergone treatment to improve their nutritional value for fish. Burel et al (2000) compared the digestibility of extruded peas, extruded lupin and rapeseed meal in rainbow trout, *Oncorhynchus mykiss* and for turbot, *Psetta maxima*. These workers found favourable digestibility coefficients for protein in thermally treated materials and this was evident for both lupin and rapeseed meals with somewhat lower values obtained for peas. A preliminary evaluation of pea seed meal for juvenile European sea bass, *Dicentrarchus labrax* by Gouveia and Davies (1998) and a subsequent study by the same authors (Gouveia and Davies, 2000) showed that substitution of fishmeal with pea seed meal and extruded de-hulled pea meal was an effective ingredient for sea bass at up to 30% inclusion in diets. Theissen et al (2003) reported favourable digestibility and growth response of juvenile rainbow trout, *Oncorhynchus mykiss*, fed with pea and canola (rapeseed) products as substitutes to soya bean meal in practical diet formulations.

The recent development of protein concentrates or protein isolates has opened new possibilities whereby the nutrient density of the diet can still be maintained by the effective reduction of fishmeal with these plant proteins. Refinements in terms of starch removal from whole pea, maize and wheat and potato results in the isolation of the protein concentrate. Maize gluten is widely used in salmon and trout diets in Europe and the USA and is a capable of replacing fishmeal provided that the lysine level of the diet is not limited. Pea proteins have also been tested for many fish but usually as the whole low protein product.

The principal strategy of this investigation was to compare the nutrient digestibility profiles of three advanced commercial plant protein rich products based on pea, maize and wheat in a short term study with rainbow trout.

Materials and Methods

Diet formulation and preparation

Experimental diets were formulated to include the plant proteins for comparison with fishmeal (LT 94), which served as the major protein ingredient of the reference diet. Each

plant protein source was substituted such that 50% of the total gross protein was effectively replaced. The pea meal protein (78% CP), Glutalys® maize protein (58% CP) and Viten® wheat gluten were products kindly provided by Roquette Brothers Ltd, France. All diets were designed to be iso-Nitrogenous (46%) and iso-caloric (21.5MJ Kg-1 DM) with the lipid and carbohydrate components maintained at similar levels. Standard vitamin and mineral premixes were included at levels appropriate for rainbow trout and yttrium oxide was the dietary marker employed.

All diets were prepared by a commercial feed manufacturer (Nutreco ARC, Norway) using a pilot extruder system resulting in low density pellets (4mm) identical in characteristics with normal commercial extruded trout diets.

Diets were stored in sealed plastic containers and kept at room temperature (20°C) prior to use.

The composition and nutrient analysis of the diets are displayed below in tables 1 & 2.

The amino acid profile of the complete diets were determined by Nutreco ARC, Norway and these are expressed as g per 100g N (% of Protein) and displayed in table 3.

Table 1. Diet formulation of diets used for digestibility evaluation.

| Ingredients | Reference | Pea protein | Glutalys® | Viten® |
|---------------|-----------|-------------|-----------|--------|
| Fishmeal | 629 | 314.6 | 314.6 | 314.6 |
| Marine oil | 125 | 153.8 | 136 | 153.2 |
| Wheat Feed | 234 | 238 | 168 | 245.2 |
| Vitamins | 6 | 6 | 6 | 6 |
| Minerals | 6 | 6 | 6 | 6 |
| Yttrium oxide | 0.2 | 0.2 | 0.2 | 0.2 |
| Pea meal | 0 | 281.6 | 0 | 0 |
| Glutalys® | 0 | 0 | 369.4 | 0 |
| Viten® | 0 | 0 | 0 | 275 |
| Total | 1000 | 1000 | 1000 | 1000 |

Table 2. Nutrient analysis of experimental diets.

| Analysis % | Reference | Pea Protein | Glutalys® | Viten® |
|------------|-----------|-------------|-----------|--------|
| Protein | 46.8 | 47.4 | 46.9 | 48 |
| Lipid | 21.5 | 22.8 | 18.7 | 22.5 |
| Moisture | 8 | 8 | 9.7 | 5 |
| Ash | 7.6 | 5.5 | 4.6 | 4.4 |

| | | | | |
|--------------|-------|-------|-------|-------|
| Carbohydrate | 13.98 | 12.78 | 14.79 | 15.3 |
| Fibre | 2.12 | 3.52 | 5.51 | 4.7 |
| Energy MJ/kg | 21.48 | 20.91 | 21.43 | 21.95 |
| Phosphorus | 1.36 | 1.16 | 0.91 | 0.80 |

Table 3. Essential Amino Acid (AA) analysis

| % AA in protein | Reference | Pea protein | Glutalys® | Viten® |
|-----------------|-----------|-------------|-----------|--------|
| Arginine | 5.03 | 6.19 | 4.28 | 4.53 |
| Histidine | 2.05 | 2.26 | 1.96 | 2.04 |
| Isoleucine | 3.56 | 3.85 | 3.53 | 3.16 |
| Leucine | 6.95 | 7.51 | 10.86 | 6.94 |
| Lysine | 6.91 | 6.86 | 4.58 | 4.60 |
| Methionine | 2.63 | 1.91 | 2.43 | 2.01 |
| Cysteine | 1.02 | 1.08 | 1.37 | 1.57 |
| Phenylalanine | 3.45 | 4.25 | 4.49 | 4.23 |
| *Tyrosine | 2.52 | 2.80 | 3.38 | 2.82 |
| Threonine | 3.98 | 3.73 | 3.64 | 3.32 |
| Tryptophan | 1.43 | 1.28 | 1.14 | 1.31 |

* Tyrosine oxidised sample.

Fish and experimental facilities

420 all female rainbow trout of average weight 110g (Hatchlands' Fishery, Devon England) were randomly assigned to 0.5 cubic meter tanks in triplicate (35 fish per tank). Each unit comprised of a re-circulation system with a constant temperature of 15°C and a photo periodicity of 14hrs light: 12 hrs dark.

Rainbow trout were acclimated to the experimental system 6 weeks prior to the start of the trial. During this period, fish were fed a standard commercial trout diet (Skretting 40) *ad libitum*.

Digestibility trial

Rainbow trout were fed the experimental diets in triplicate to satiation in two daily meals (morning and late afternoon) for 4 weeks prior to faecal collection. Fish were fed at approximately 1600-1700 hrs prior to the following days stripping period. The faecal collection by stripping was the preferred method and avoids leaching of nutrients. This is

the technique described by Austreng (1978). Each rainbow trout was lightly anaesthetised with MS222 and mild pressure was applied to the abdomen for expelling faeces into a foil container. Faeces were processed immediately drying wet material at 75°C in a convection oven until constant dry matter was achieved and ground to a fine powder.

Chemical analysis

Diets and faeces obtained from each group of fish were all subjected the same gross nutrient analysis as defined by AOAC (1990). This included gross protein by Kjeldahl, lipid by Folch and energy. Carbohydrate was analysed by a direct enzymatic method for available carbohydrate as Starch (Megazyme Ltd, Ireland). Amino acid profiles were determined by Nutreco ARC, Norway. Yttrium oxide and calcium & Phosphorous were measured by ICP-ES after wet acid digestion of samples in Nitric acid within a bomb microwave apparatus.

Digestibility calculation

Digestibility of the nutrient components in diets were calculated according to equation (1) and the respective ingredient by the ratio of test ingredient contribution and reference diet as stated in equation (2). These are described by Lupatsch et al (1997) as applied to sea bream and established for many other fish species in aquaculture.

Formula 1:

$$\text{ADC (\%)} = 100 - [100 \times (\text{Y}_{\text{food}} / \text{Y}_{\text{faeces}}) \times (\text{Nutrient}_{\text{faeces}} / \text{Nutrient}_{\text{food}})].$$

(Y and nutrient in g kg⁻¹)

Formula (2)

Partial digestibility coefficients were calculated using:

$$\text{DC}_T = [\text{DC}_D - (\text{DC}_r \times r)] / t$$

Where DC_D is the digestibility coefficient of the nutrient in the diet (%); DC_R is the digestibility coefficient of the nutrient in the reference ingredient (%); DC_T is the digestibility coefficient of the nutrient in the test (%); r is the contribution of the nutrient of the reference ingredient to the diet (%); and t is the contribution of the nutrient of the test ingredient to the diet (%).

Statistical evaluation of data

Where appropriate, mean values of triplicate groups of fish (n=3) are reported and Standard Errors included. Data was tested by ANOVA at the $P < 0.05$ level of significance.

Results

Rainbow trout readily consumed the experimental diets and there were no adverse effects or palatability constraints associated with the use of pea, maize or wheat derived protein ingredients at the levels tested.

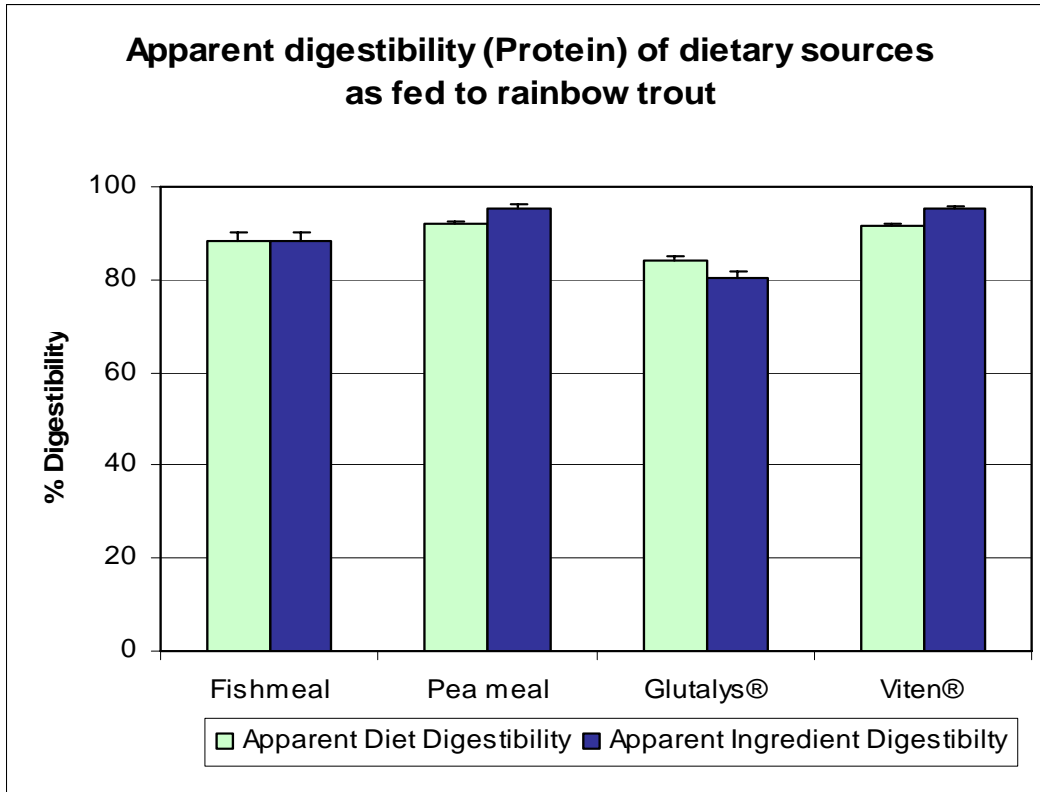


Fig. 1 Apparent digestibility coefficients for protein (dietary and test ingredient values) as fed to rainbow trout

On the basis of the proximate analysis and relative yttrium oxide of diets and faecal material, it was possible to obtain nutrient coefficient data for each of the test ingredients within the experimental diet formulations. Fig.1 shows that the apparent crude protein digestibility coefficients of the fishmeal and test protein sources. The digestibility of fishmeal (LT 94) was in the order of 90% with pea meal concentrate having a higher protein ingredient digestibility value of 95.5% and similar in magnitude to Viten® (95.24%). A lower value of 80% was obtained for the ingredient protein digestibility of Glutalys®.

The essential amino acid (EAA) digestibility profile followed a similar trend to protein digestibility as shown in figure 2. The highest value was for lysine and arginine (99%) with most values above 90% for EAA's with the notable exception of EAA's within Glutalys® at less than 90%.

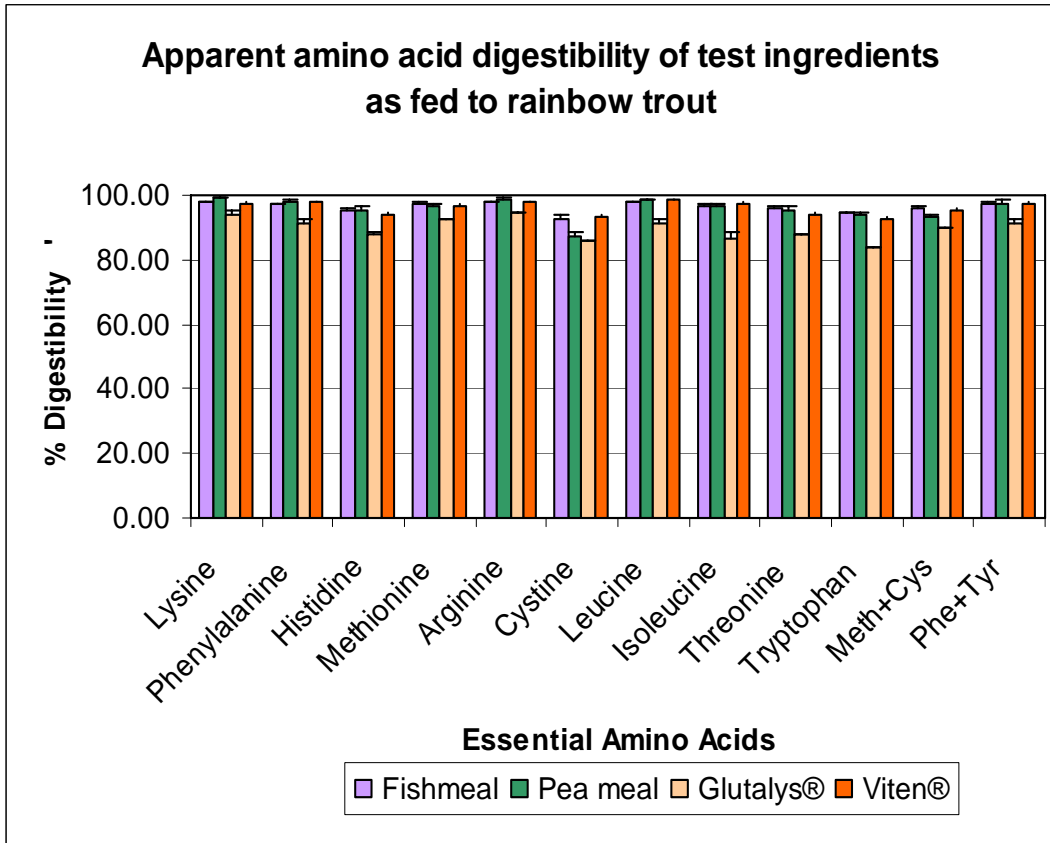


Fig. 2 Amino acid digestibility coefficients of test ingredients fed to rainbow trout

Apparent lipid digestibility was also reported for the test diets (fig. 3) and this appeared to be slightly reduced by pea meal substitution to 94% compared to 96% for fishmeal fed trout.

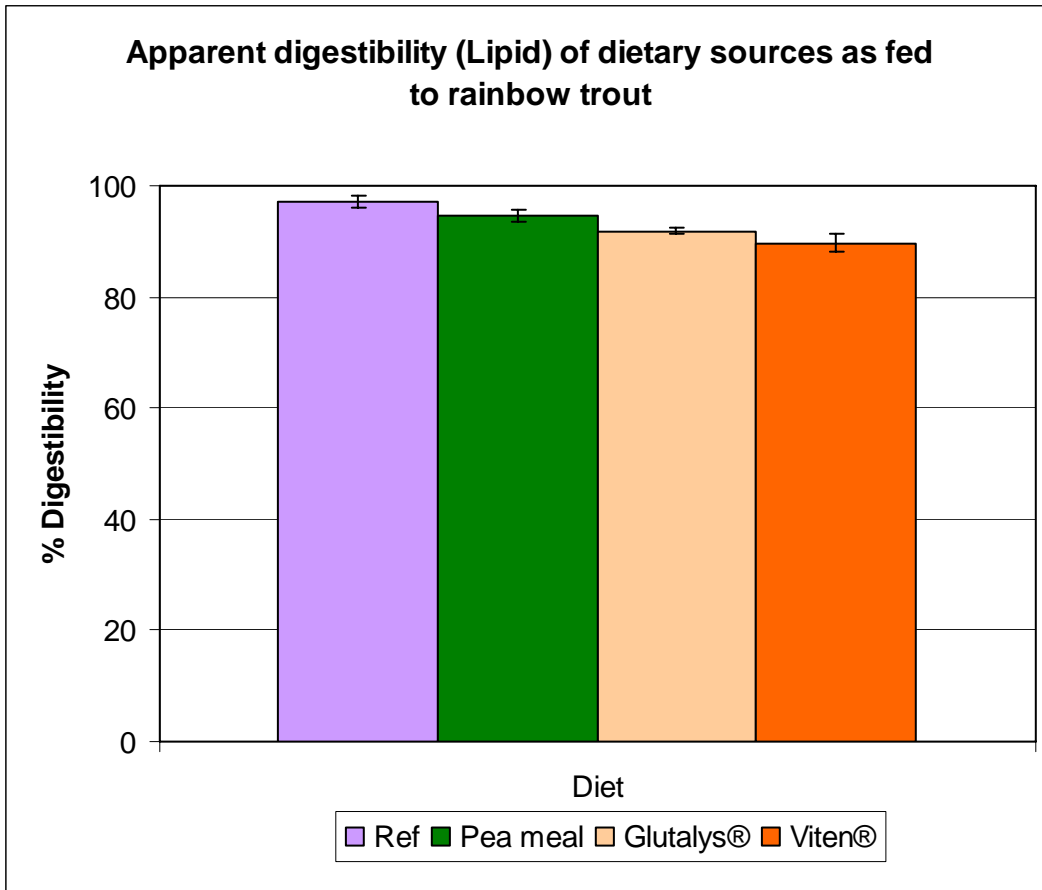


Fig. 3 Apparent lipid digestibility of dietary sources as fed to rainbow trout

Incorporation of both Glutalys® and Viten® cereal derived protein concentrates appreciably reduced the lipid digestibility to a value of ~ 90%.

Carbohydrate digestibility as determined directly as starch availability within the experimental diets, are presented in figure 4. There was a pronounced effect on the carbohydrate digestibility for rainbow trout receiving diets containing Glutalys® and Viten® products. The apparent digestibility coefficients were 80% for Glutalys® and only 75% for Viten® compared to 82% for the carbohydrate in the fishmeal reference group of trout. Interestingly, starch digestibility was elevated for the pea meal based diet at 85%.

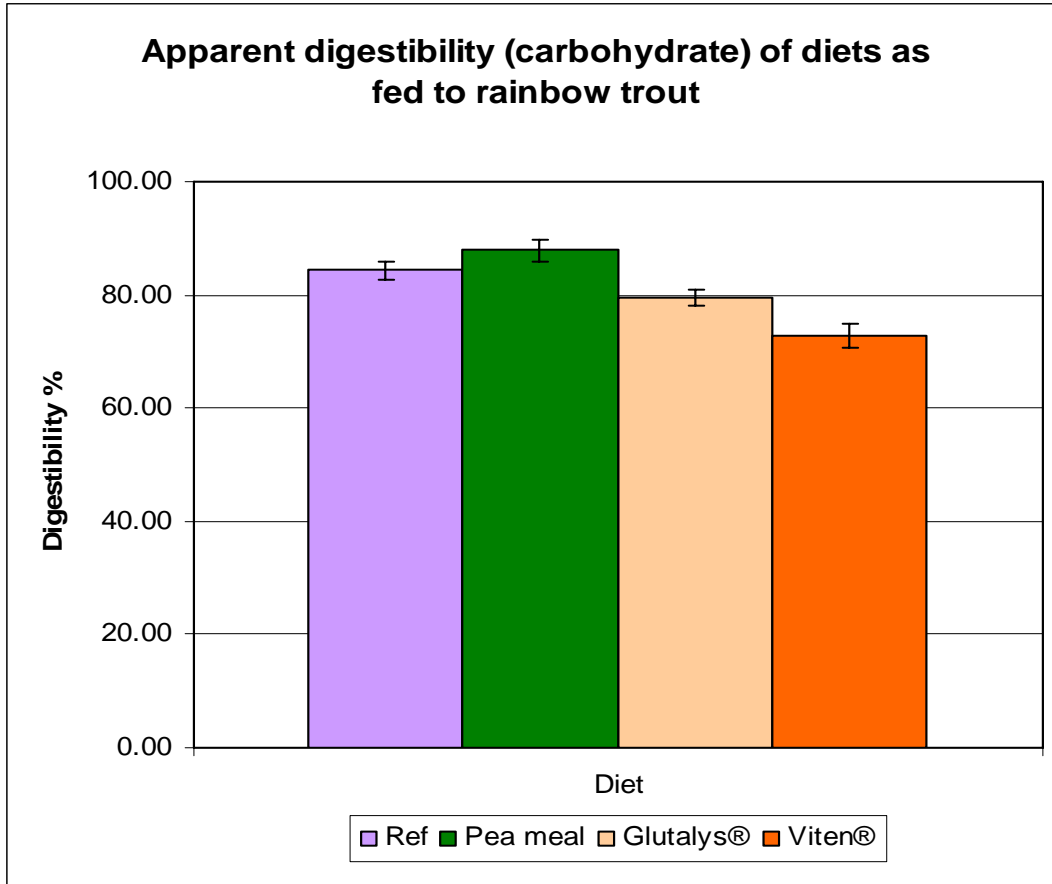


Fig. 4 Apparent digestibility of carbohydrate in dietary sources as fed to rainbow trout

Energy digestibility was over 80% (Fig.5) for the LT fishmeal and for the pea meal concentrate respectively. However this was reduced to 70% for the Glutalys® fed group of trout and was below 70% for rainbow trout fed the Viten® product.

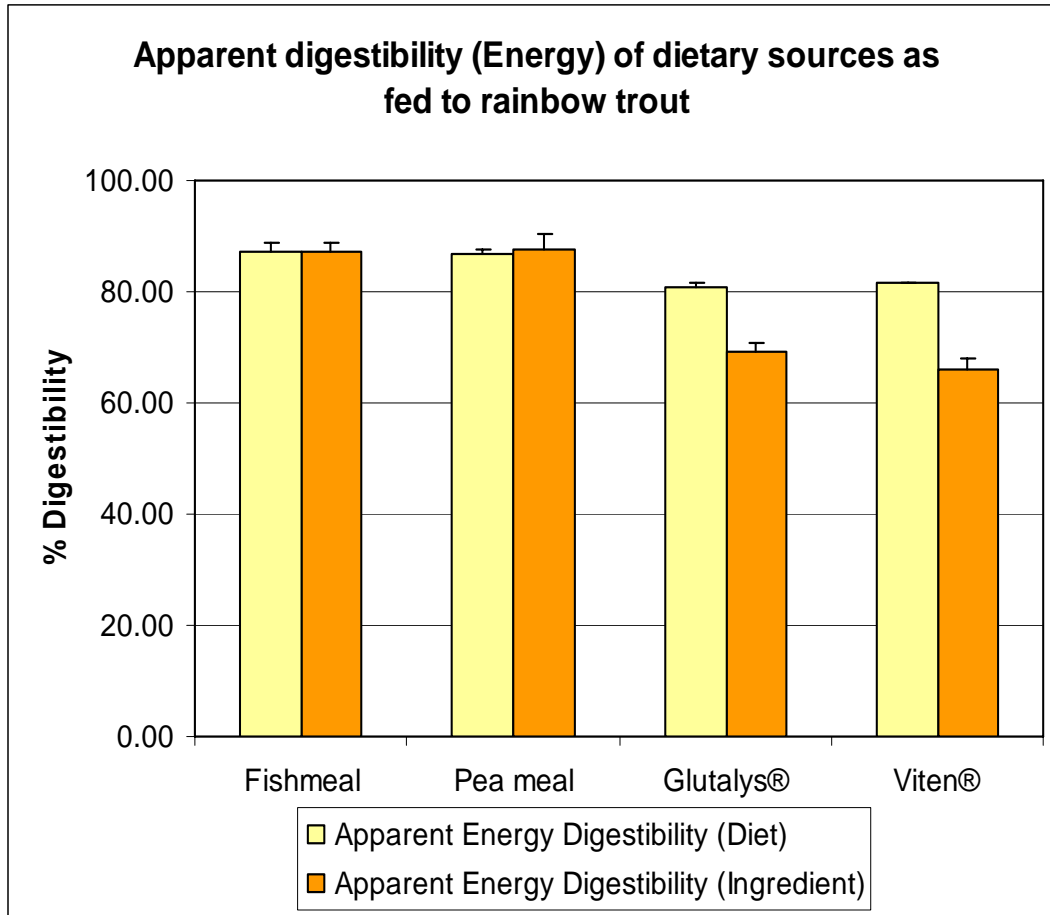


Fig. 5 Apparent energy digestibility of dietary sources & test Ingredients as fed to rainbow trout

The apparent phosphorus mineral availability coefficients were also measured and these are shown in Fig. 6. It is evident that no obvious negative effects on P absorption results from the inclusion of the products tested. Indeed P absorption was slightly elevated compared to the value obtained for the fish meal diet. These ranged between 52-58%. Ca values are difficult to assess due to the fish’s ability to absorb the element across the gills. Calcium values varied from positive to negative coefficients. The calcium result has been displayed for reference in figure 7.

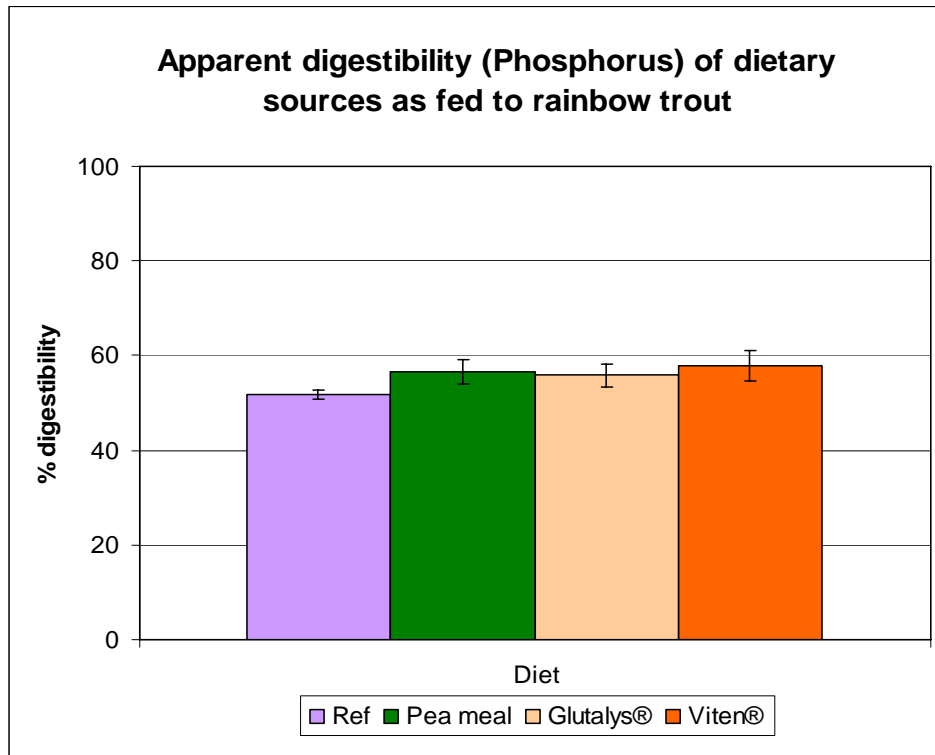


Fig. 6 Apparent phosphorus availability of dietary sources as fed to rainbow trout

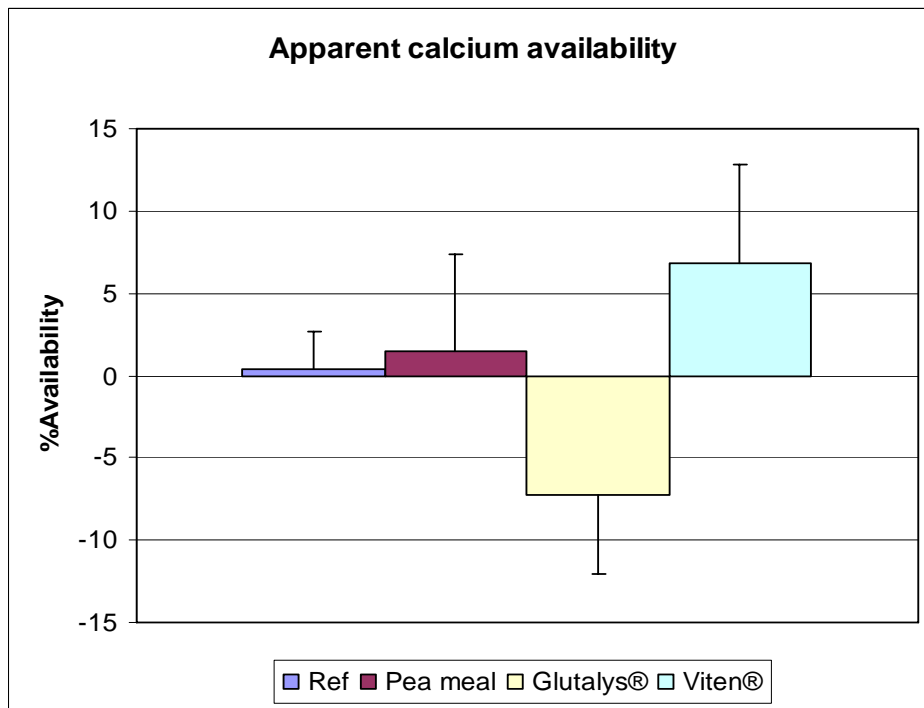


Fig. 7 Apparent calcium digestibility for each dietary treatment

Discussion

Preliminary evaluation of novel feed ingredients for animal nutrition is best served by undertaking short term digestibility trials to assess their potential nutritional value on the basis of establishing reliable nutrient digestibility coefficients. Most studies with fish however tend to focus on gross nutrient substitution in experimental diets fed over a long period of time to determine growth and feed utilisation parameters. Optimum diet formulation depends upon accurate data of the digestibility of protein and also the individual essential amino acids. The present investigation provided such information for the protein in selected commercial protein sources namely pea, maize and wheat protein concentrates.

Akiyama (1991) stated the importance of defining the availability of nutrients in determining the nutritional value of feed ingredients in a review on the use of soy products and other plant protein supplements in aquaculture feeds. The approach used in the current investigation with rainbow trout is the classical technique adopted by many workers based on the use of incorporating a fixed proportion of the ingredient within a reference diet. However many studies fail to adequately balance the diet formulations in order to maintain a set level of protein and energy in the mixed diets. In this study, the test ingredients were included to replace 50% of the fishmeal and lipid and carbohydrate levels remained the same, since digestibility is known to be affected by the ratio of energy to protein. There is also the question concerning the use of the digestibility calculation and Forster (1999) advocates a refinement to allow for specific nutrient ratios. This approach was employed in the present study with rainbow trout.

The protein digestibility values obtained in the trials were of similar magnitude as reported by other researchers evaluating a range of different plant ingredients. Burel et al (2000) found fairly high digestibility for protein in extruded peas, lupins and rapeseed meals for trout and turbot. However they did not provide any amino acid availability coefficients and the test ingredients were all included at 30% with marked variations in the final crude protein levels of diets.

A majority of established data for fish is presented for soyabean meals of varying quality that differ in their degree of processing and complexity.

Conventionally, dehulled solvent- extracted soybean meal is the most commonly used plant protein ingredient in commercial fish diets and is now widely used in salmonid aquaculture feeds. Olli et al (1995) showed that the growth and feed utilisation performance of rainbow trout could be upheld when soyabean meal was included at 20% of the diet but that these parameters were appreciably reduced above 40% inclusion. Evaluation was based on gross protein substitution only and no allowance or data was obtained for differences in digestibility at each inclusion level.

Oliva-Teles et al (1994) investigated the effect of different processing treatments on soyabean meal utilization by rainbow trout in a 12-week feeding trial that also included a digestibility trial using automated faecal collection. In these studies, no significant benefit was found between soyabean meals that had been thermally treated. However at 20% incorporation at the expense of fishmeal protein, all plant diets were superior to the fishmeal reference diet with respect to performance. It should be cautioned that they did not validate the source of fishmeal in their studies and in our experiments with trout, a premium grade fishmeal (LT94) was used. The use of inferior grades of fishmeal can compromise the findings of studies to evaluate the comparative performance of alternate proteins resulting in over optimistic conclusions. Typical values for soybean meal protein digestibility range from 80-95% depending on processing, and serve a useful reference for these trials with protein concentrates.

Corn gluten is an interesting protein source for fish and is relatively low in anti-nutritional factors and is used in both salmon and trout commercial diets.

Pereira and Oliva-Teles (2003) reported separate digestibility coefficients for corn gluten meal (CGM) in investigations with Gilthead sea bream, *Sparus aurata* and found that protein digestibility was only affected when the material was included at 80%. They concluded that CGM could be used in diets for this species at levels up to 60%, as

confirmed by nutritional trials to study growth performance. Previous work undertaken by Wu et al (1995) testing corn gluten meal for tilapia, *Oreochromis nilotica*, found that partial substitution of fishmeal with CGM supported good growth and feed conversion.

The protein and amino acid digestibility profiles of maize gluten meal indicated reasonable availability but lower than fishmeal. This has also been seen in other experiments with fish and may be due to the higher viscosity of the material and interaction with starch.

A few investigators have tested the feasibility of wheat gluten type products for fish with variable results. The principle reason was mainly to determine the lysine requirements of fish since this protein is deficient in this particular essential amino acid. Pfeffer and Henrichfeise (1994) reported that rainbow trout had a marked depression in growth when dietary fish meal was replaced by wheat gluten meal but was greatly improved when the diets were supplemented with L-lysine. Similarly, Davies et al (1997) effected the partial substitution of fish meal and full fat soybean meal with wheat gluten and the influence of lysine supplementation. This latter study was based on the full amino acid profiles and accounted for the digestibility of lysine in the test diets. The present experiments with rainbow trout resulted in similar digestibility coefficients for lysine with the Viten® product and confirmed the high protein and EAA availability of a wheat protein for this species.

Considerable interest in pea protein sources results from its more balanced amino acid pattern with only methionine being the first limiting amino acid. Disadvantages are mainly due to the low protein and high starch content of whole pea meal products.

The use of pea protein flour with a crude protein of over 80% is a distinct improvement and allows higher substitution of fish meal proteins in diets in nutrient dense diets for fish.

The opportunities and constraints of utilising feed peas and chick peas in aquaculture diets were reviewed by McCallum (1997). Indeed the promising role of pea meal by-products in diets for marine fish was presented by the work of Gouveia and Davies (1998; 2000) in experimental trials with sea bass. Digestibility coefficients were obtained

for protein, lipid and energy and no deleterious effects noted when a whole pea meal was included at up to 30% of the diet. A dehulled pea meal product was also shown to be an effective ingredient for these fish. Higher inclusion levels could not be evaluated by these researchers due to the spatial limits imposed by the bulk density effects of the product and low protein content. Olvera-Novoa et al (1997) however were able to test the value of a cowpea protein concentrate for tilapia and stated that the best performance in terms of growth, survival and feed utilisation efficiency was at the 40% inclusion level.

There has been limited work undertaken with salmonid species, and for juvenile rainbow trout, Thiessen et al (2003) found excellent digestibility for pea seed meal protein for raw/whole peas, raw/de-hulled peas, extruded/de-hulled peas and autoclaved air-classified pea protein in experimental practical type diets. Digestibility of the protein component was uniformly high for all pea ingredients (90.9-94.6%), regardless of the processing method employed and supports strongly the results described in our study. In general, nutritional assessment confirmed that 20-25% pea meal was feasible for trout in feeding and growth studies by these latter workers.

Unfortunately no amino acid availability data for pea products was provided by any of these authors for the fish species tested. In the present investigation with rainbow trout, excellent protein and essential amino acid digestibility coefficients were recorded for the pea protein flour including methionine despite the slightly lower contribution in the complete diet.

Protein and amino acid digestibility is known to be affected by the interaction of other nutrient components in the diet. It is evident from the trials with rainbow trout that lipid and carbohydrate digestibility was reduced by the incorporation of both maize and wheat gluten products. The digestion of dietary starch will depend on its relative complexity (Gouveia and Davies, 2004 in press) and physical characteristics will vary in the pea, maize and wheat sources after extrusion. The degree of gelatinisation may alter the viscosity of the diet and also influence lipid digestibility. This might explain the results found with trout since the pea meal concentrate containing little starch had only a slight effect on lipid digestion and actually resulted in a better starch digestion. These trends would help explain the energy digestibility coefficients for the different diets.

Another major concern with the choice of plant proteins is the bio-availability of phosphorous bound in the ingredient. Plant ingredients such as soyabean meal contain most of the phosphorous as phytate, and are largely unavailable to the fish when present in the diet (Spinelli et al 1983). This would cause reduction in the absorption of supplemented inorganic phosphorous as well adding to the pollution burden and environmental impact of fish feeds such as those for salmon and trout. Fortunately, our studies with rainbow trout indicated that phosphorous availability was higher for all the plant protein concentrates, but this may have been due to a reduction in the ash levels of diets due to the reduction in the fishmeal component.

In conclusion, it would seem that on the basis of a preliminary digestibility trial, pea protein was a consistent product with a very good nutrient availability for rainbow trout. Although the Viten® was also favourably utilised, Glutalys® had an overall inferior digestibility profile for protein and energy. It would be imperative to consider further evaluation of these products based on nutritionally balanced diets for rainbow trout using the data obtained here for formulations based on digestible protein, essential amino acids and energy. It may be necessary to consider supplementation with methionine, lysine, tryptophan and threonine in particular cases. Future studies could also examine complementary use of blended ingredients in complete diets resembling commercial feeds and trials should also be extended to other fish of economic importance especially marine fish species such as sea bass, bream and turbot.

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The work conformed to the 1986 Scientific Animal Procedures Act (UK) and the University of Plymouth Bioethical Committee.

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