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165551 OLD COLONIAL ROAD
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PROJECT TITLE:

'REDUCTIONOF FISHMEAL IN COMMERCIALLY IMPORTANT TEMPERATE /MARINE FISH SPECIES WITH SELECTED ANIMAL PROTEIN CONCENTRATE BLENDS WITH RESPECT TO PROTEIN, ENERGY AND AMINO ACID DIGESTIBILITY'

PRINCIPAL INVESTIGATOR: Dr Simon J Davies, Fish Nutrition Unit, School of Biological Sciences, University of Plymouth, Drake Circus, Plymouth, UK PL48AA
Co- Investigator: Robert D. Serwata

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Introduction:

Of serious concern is the sustainability of aquaculture due to the dependency of intensive rearing on complete balanced diets based mainly on fish meal and fish oil. Fish meal is the principal protein source and demand in future will impose severe constraints on this precious resource derived from the marine environment
It is therefore imperative that research is directed to realizing the potential of replacing fish meal with alternative sources. Although vegetable proteins such as soyabean meals and by-products obtained from pulses and legumes are feasible for some fish species, there are many problems due to the presence of anti –nutritional factors and toxins that limit their potential for carnivorous fish especially. There is much scope therefore for animal proteins that are free from such factors and also benefit from higher digestibility and an amino acid profile closer to the requirements for these fish.
Fish nutrition investigations in Europe have been hampered as a result of legislation and public concerns regarding TSE's and the negative image of animal proteins in feeds and the food chain.
The new generation of selected high quality animal proteins presents a promising future and need urgent evaluation for future applications in marine fish farming. Standard nutritional trials require fish meal substitution with test proteins and fish performance measured in terms of growth and feed conversion parameters.

It is preferable to first undertake a comprehensive series of short term digestibility experiments to grade the characteristics of animal proteins prior to conducting costly growth and balance trials with different fish species. This was the basis for the investigations described here for research supported with FPRF.

The digestibility trials were undertaken as a preliminary nutritional assessment of a comprehensive selection of animal by products and various blends based on products commercially available in Europe.
These were provided by Prosper de Mulder Group in consultation with technical specialists that provided characterisation of there nutritional profile. Given that the Fish Nutrition Unit at Plymouth is currently involved with similar trials for rainbow trout and Tilapia it was deemed appropriate to extend investigations to cover marine species found in the Mediterranean. The species of choice included sea bass and turbot as prime examples of fish cultured in Southern Europe for the aquaculture market.
Earlier work by (Nengas et al, 1999) in association with the Plymouth unit was able to verify the potential of animal by products for use in sea bream, limited digestibility data was available and experiments were focused on longer term nutrition trials.
There is a paucity of information concerning the bio-availabilities of major nutrients for fish with respect to most of the protein rich ingredients used. It is now recognised that before commencing nutrition/feeding trials, it is better to attempt to establish the full digestibility profile of potential ingredients in short term digestibility trials.
Objectives:

The main objectives of the separate investigations were to obtain reliable digestibility coefficients for a series of test ingredients within experimental diets that may be used for

sea bass and turbot diets in the future. The trials were designed to focus on the digestibility coefficients of protein and amino acids in particular for the selected ingredients. Where appropriate the digestibility of lipid and energy are reported for the experimental diets.
The main protein sources employed were fishmeal (control), feathermeals (standard and enzyme treated) poultry meat meal and a haem protein concentrate. A number of appropriate blends of these ingredients were also evaluated as potential replacements for fishmeal.
The principal aim is to establish such coefficients for determining which ingredients may be most appropriate for use in balanced diet formulations that could follow from the preliminary digestibility trials.

Industry summary:

Trials were conducted with both sea bass and turbot using diets in which fishmeal was the basal protein within a reference diet. This diet was substituted at 40% for sea bass and 30% for turbot using the individual test ingredients namely steam hydrolysed feathermeal, enzyme treated feathermeal (Allzyme), poultry meat meal, spray dried haem. The blends were a 75% test ingredient / 25% spray dried haem mixture; the following blends were manufactured steam hydrolysed feathermeal & spray dried haem, enzyme feathermeal & spray dried haem, poultry meat meal and spray dried haem.
The concept of including the test ingredients/blends at a defined inclusion against fishmeal in separate experimental diets allows a realistic assessment of digestion and hence digestibility in diets that resemble commercial situations. Trials with sea bass and turbot were very successful and for each species diets were all accepted by the fish and we were able to measure the digestibility of each of the selected nutrient parameters for the products. The findings confirmed that our expectations that some animal proteins would perform better than others and be comparable to our previous findings with rainbow trout.

The following ingredient sources compared favourably with fishmeal for protein, amino acids and energy under the conditions of the trials; for sea bass (spray dried haem, poultry meat meal & the poultry meat meal / spray dried haem blend). The following ingredients performed slightly less favourably for sea bass (steam hydrolysed feathermeal, enzyme feathermeal and the blends of these previously mentioned ingredients).
The following ingredient sources compared favourably with fishmeal for protein, amino acids and energy under the conditions of the trials; for turbot (spray dried haem, and the blends with spray dried haem with poultry meat meal, steam hydrolysed feathermeal and enzyme feathermeal). Interestingly poultry meat meal did not perform as well for turbot as in sea bass although the variations were highest for this product. The lowest results of digestibility for protein were obtained for both types of feathermeal as tested on turbot.
It is quite realistic to assume that specific ingredients would not be included at levels as high as those needed for a digestibility trial infact it might be assumed that lower levels would be used and found as blends to substitute fishmeal in practical diets for sea bass

and turbot. In this way levels of 10-30% would be encountered typically in linear least cost formulations. The digestibility coefficients of protein, amino acids, and energy could be used for these ingredients to provide a more sensitive diet formulation in order to substitute the protein and energy components more accurately. The cost per unit of protein would be included in the analysis and linear least cost formulation could then be used to produce a range of experimental diets for longer term nutrition trials.

Reduction of fishmeal in temperate marine fish species (sea bass & turbot) using selected animal protein concentrates and blends with respect to nutrient digestibility profile.

S.J Davies[1]*, R.D Serwata[1], Laporte[1], J., A.Gouveia[2], S.L Woodgate[3]

[1] *Fish Nutrition Unit*, School of Biological Sciences, Portland Square, University of Plymouth, PL4 8AA, UK. Email: sdavies@plymouth.ac.uk

[2] Faculdade de Ciências, Universidade do Porto, 4099-002, Porto, Portugal

[3] Prosper de Mulder Group, Greenleigh, Kelmarsh Rd, Clipston Mkt. Harborough, LE16 9RX, UK

Abstract

Two experiments were conducted using sea bass (*Dicentrachus labrax*) and turbot (*Petta Maxima*) in seawater under controlled temperature and photo period. A selection of animal by products from commercial sources were substituted for fishmeal at a fixed inclusion rate appropriate for each species to evaluate the coefficients of digestibility for crude protein, essential amino acids and dietary energy and dietary lipid respectively.

These were short term trials that followed an acclimation period when which the fish were fed the experimental diets to satiation. Feacal material was recovered at the end of the feeding stage and nutrient digestibility profile related to the measurement of chromic oxide as the inert marker.

Results confirmed that certain products were well digested by these species and a Spray Dried Haem protein (SDH) performed as well as a low temperature (LT) fishmeal. For sea bass, Poultry Meat Meal (PMM) was particularly good with respect to protein and energy. Steam Hydrolysed Feathermeal (SHF) and an Enzyme Treated Feathermeal (ETF) were only adequately digested for protein and energy. A blend of PMM & SDH made no appreciable benefit for sea bass, however the same blended proteins were better utilised for turbot compared to poultry meat meal alone.

Keywords:

Sea bass, turbot, animal by- products, digestibility, protein, essential amino acids, energy.

Introduction

A decade ago, in 1994, global aquaculture production of aquatic animals was only 12-million Mt. Over 50 percent of seafood production originated from inland waters (whilst only 10% of seafood production came from aquaculture enterprises in marine waters). Global aquaculture production is now expanding by an average rate of 14 percent per annum. Total production is amounted to almost 22 Million metric tonnes by 2000 (New, 1991). Indeed, aquaculture must increase by nearly 400 percent if the expected deficit of 60 Million tonnes is to be realized for the total 162 M Mt demand for seafood in 2025 (Hardy and Kissil, 1996)

Aquaculture production of high value species has the potential to increase much further (Fridley, 1993), especially in marine locations. As well as the production of mainly Atlantic salmon, *Salmo salar* in Norway, Scotland, North America and Chile, considerable output of Gilthead seabream, *Sparus aurata*, sea bass, *Dicentrachus labrax* and turbot, *Scophthalmus maximus* exists in the Mediterranean region.

Numerous studies have been directed towards establishing the nutritional value of animal and plant proteins for fish and in particular, salmonid species such as trout, salmon and some fresh water fish species (Higgs et al.1997; Gouveia, 1992; Pfeffer & Henrichfreise,1994; Allen et al. 2000; Davies, 2002) however to date little research has been performed on marine fish of commercial aquaculture importance, apart from previous work with Nengas et al (1999) to test the feasibility of high inclusion levels of poultry meals and related by-products in diets for the gilthead sea bream, *Sparus aurata.* Although recently Millamena & Gomez (2002) evaluated processed meat solubles, as a replacement for fishmeal in diets for grouper *Epinephelus coioides* with good performance.

There is clearly a growing need to obtain reliable information in order to assess the potential of novel feed ingredients for the replacement of fish meal in diets for fish in general due to the sustainability problems associated with the expanding aquaculture industry. The dependence on fish meal as the primary protein source will be a major issue and has been recognised by government agencies and the industry at large. Obviously, plant proteins are a distinct possibility for many fish and have been studied in detail for

carnivorous fish as well as omnivore's species (Tacon, 1994). The high value carnivorous fish offer less scope for the utilisation of plant proteins and therefore the use of animal proteins becomes more realistic. The recent developments in the technological advances of processing animal proteins provide a new opportunity to address their inclusion in fish diets (Woodgate, 1996; Bureau et al. 2000).

Research to define the potential for poultry by-product meal, feathermeals and blood meals together with various blends and mixtures has been undertaken by the authors with rainbow trout, but work was needed to test these products with marine fish species of relevance to Europe. European sea bass and turbot were the fish of choice and were the basis of the experimental studies. The materials provided were typical standard commercial products used widely by the industry in Europe with some products representing new materials based on considerable technical advances in feed processing.

Materials and Methods

Fish and facilities

The experiments conducted were undertaken on commercially farmed sea bass (mean weight 100g) and juvenile turbot (mean weight 100g). These were purchased from Aguarela-Sociedade de Piscicultura, Lda, Aveiro, Portugal and transferred into the quarantine unit of the research aquarium prior to grading. Fish were then acclimated to the experimental holding systems for 2-weeks and fed a medicated feed formulated for marine fish species to ensure pathogen free fish were ready for the test diets as described above.

The water temperature was held at 24°C±1°C with a salinity of 33-34ppt. The Photoperiod was maintained at: 12 h light; 12h dark by means of artificial daylight simulation. Each diet was tested in triplicate and sea bass were randomly assigned (15 per tank unit) to cylindro-conical tanks of the following dimensions: length-40cm; width-17.5cm; depth- 27-38cm

The tank volume was 60L and a flow rate of seawater resulting in a complete exchange of 5 volumes per hour. The tanks were designed to be based on the Guelph system and faeces were collected daily after thorough cleaning of the Perspex traps following each evening ration.

Feeds and feeding

A series of experimental diets were formulated for sea bass and turbot as shown in table 1&2 in which fishmeal (LT-94) was the primary constituent protein. The animals by products were included to replace 40 and 30% of the protein for the reference diet. Lipid levels were adjusted in order to achieve caloric balance. The test ingredients were Steam Hydrolysed Feathermeal (SHF), Enzyme Treated Feathermeal (ETF), Poultry Meat Meal (PMM), Spray Dried Haem (SDH) and blends that consisted of the following (75% SHF/25%SDH), (75% ETF/25% SDH), (75% PMM/25%SDH).

The technical characteristic of the materials are based on specifications provided by the relative manufacturers. Poultry by-products were provided by Prosper De Mulder Group, Market Harborough, England.

Steam Hydrolysed Feathermeal was a mixed poultry feather source hydrolysed at 5.5 bars pressure for approximately 30 minutes. This was dried by an indirect steam drier (Rotadisc) to ~5% moisture. Enzyme Feathermeal was heated to 50C in presence of an enzyme and mixed for 30 minutes. Hydrolysis was followed by processing at 2 bars pressure for 15 minutes and steam heated to ~5% moisture. Poultry Meat Meal was form mixed poultry sources deemed fit for human consumption. The material was minced to <3mm introduced into a continuous process (Rotadisc) to evaporate water, sterilize in presence of natural fats. The residence time is about 90 minutes with a maximum temperature of 125 deg C. The resulting material is concentrated by an expeller press to remove fat. The protein rich fraction is cooled and milled. The haemoglobin (Haem Protein Concentrate) was manufactured by American Protein Corporation (APC) Des Moines, Iowa, USA. The AP301 product is whole porcine blood, from animals slaughtered fit for human consumption. The blood is chilled and separated into plasma and red blood cell fraction (Haemoglobin). The latter is spray dried to produce a dry (<5% moisture) Haemoglobin powder.

Diets were prepared using a California Pellet Mill (CPM) in which all dry ingredients, vitamins and mineral premixes were uniformly mixed together before the addition of marine fish oil and de-ionised water. The resulting mixture was extruded through a 4mm aperture die and the resulting pellets air dried by convection until moisture content was <10%. The diets were all stored in plastic sealed containers and frozen prior to there use in the trials.

The test diets were administered three times daily and fish were fed to near satiation. This resulted in a feeding level close to 1.5 % per day over the period and fish accepted the test diets well. The fish grew by approximately 25% during this time and were therefore in positive balance. There were no obvious signs of stress or adverse effects of diets.

Excess feed was removed daily from faecal traps before commencement of faecal collection.

Faecal material was pooled for each triplicate group within each tank containing sea bass or turbot separately and dried to constant weight in an oven at 105°C. This was subsequently ground to a fine powder and stored in air tight plastic containers until analysis for nutrient components and inert marker.

Diet and faecal analysis

All diets were subjected to proximate composition analysis according to AOAC methods for crude protein, energy, ash and moisture. The exception being lipid analysis in which a modified Folch technique was employed according to the following protocol, 1.5g samples were subjected to a (1:1) 10ml methanol/ 10ml 6N HCL extraction (30 minutes in an oven @70°C) samples were then allowed to cool to room temperature. A further addition of dichloromethane (20mls) was added. The tube was capped and thoroughly shaken for 20 seconds and left to stand for 30 minutes. Samples were then centrifuged for 10 minutes at 3000rpm. The top layer of methanol and water was removed and the subsequent hypo phase was removed (2.5mls) with a gas tight syringe. Approximately 2mls of the hypo phase was placed into a pre-weighed glass vial. The dichloromethane was evaporated using a gentle flow of nitrogen. The samples were then further oven dried to constant weight and placed in a dessicator before the final vial weights were determined.

Prior to amino acid quantification samples were subjected to 6N HCL hydrolysis for 24h in sealed glass ampoules, for tryptophan analysis the samples were subjected to 4N Methane Sulfonic acid hydrolysis for 16 hours.

Amino acid analysis was undertaken using a Dionex Electrochemical Detector following chromatographic separation.

Chromic oxide was measured following nitric & perchloric acid wet acid digestion and colourimetric detection by visible spectroscopy according to the method of Furukawa and Tsukahara (1966).

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.

Formula 1:

$$ADC(\%)=100-[100x\ (_{food}\ /\ Cr_2O_{3\ faeces})$$

$$X\ (Nutrient_{faeces}/Nutrient_{food})].$$

(Cr_2O_3 and nutrient in g kg^{-1})

Formula (2)

Partial digestibility coefficients were calculated using:

$$DC_T=[DC_D-(DC_r\ x\ r)/t$$

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

(Trial 1, sea bass)

The results for sea bass numerically presented in table 3. These indicate a particularly good availability of protein for poultry meat which was above 80% digestible compared to 93% for the fishmeal in the reference diet (as single ingredients). Both feathermeal and enzyme treated feather meal did not differ greatly in their digestibility (DC) for protein being approximately 75% digested for sea bass. Spray dried haem as a single ingredient gave a high digestibility coefficient for protein which was close to 90%. However a mixture of feathermeal and spray dried haem as a blend had a reduced digestibility for protein of 65%. The enzyme feathermeal spray dried haem blend however resulted in a slightly higher digestibility of 72%. The combined poultry meat meal and spray dried haem resulted in a protein digestibility of over 80%. It was apparent therefore that poultry meat meal and spray dried haem either as single ingredients or as a blend offer considerable potential for this species in diet formulations for this species. The protein digestibility profile for the different diets and ingredients are also presented in figure 1. The relative essential amino acid digestibility data for sea bass are shown in figure 2. It is evident that the essential amino acid digestion pattern reflects the overall digestibility (DC) of protein with however some fundamental differences with respect to specific amino acids. In particular, it was seen that the DC of cysteine in feathermeal was appreciably lower for both types of material evaluated compared to 95% for fishmeal but lower for the enzyme processed product (62.07%, 57.42% respectively). Interestingly, methionine was also lower for the enzyme treated feathermeal compared to the standard steam hydrolysed product (87.93% v 99.90%). Histidine digestibility was however markedly improved as a result of enzyme processing of feathermeal. Lysine digestibility was good for most ingredients (96.71% for fishmeal) with over 97% for poultry meat meal (PMM). Indeed PMM showed consistently high DC values for all important amino acids i.e. phenyl alanine, histidine, arginine, leucine, isoleucine, threonine and tryptophan. Spray dried haem was also uniformly high in the DC profile of these amino acids. Although, cysteine was over 77% digestible for spray dried haem compared to only 64% for poultry meat meal for this species. Generally, blended proteins were well digested with respect to their EAA characteristics. The poultry meat meal: spray dried

blended combination especially resulted in DC values of 85- 100% for most EAA's with the exception of cysteine (49.93%).

Digestible energy values for the combined diets ranged from 53% to 82% (the lowest obtained for feathermeal and spray dried haem blend). The highest was for the poultry meat meal and spray dried haem complement. These values demonstrate the variations that occurred reflected mainly the digestibility coefficients for the protein and the lipid of each diet. The lipid digestibility values ranged between 61% - 82% for the diets, except for the hydrolysed feathermeal and spray dried haem blend which produced a negative value implying accumulation of lipid in the faeces. This was repeated in triplicate tanks and will require evaluation.

(Trial 2, turbot)

The similar experiment for turbot has produced interesting data comparable in many respects to the sea bass trial. The numerical digestibility parameters for turbot are shown in table 4. Protein digestibility (DC) for specific ingredients, are generally lower than for sea bass, this included the reference diet containing fishmeal. These are also shown in profile in figure 3.

Protein digestibility for spray dried haem was the highest at 80%. Poultry meat meal was 65% digested both feathermeal (hydrolysed and enzyme treated) were digested to a similar degree at 61%. Interesting, for turbot combinations of both feathermeals with spray dried haem were slightly better digested with values of 68.6% and 69% respectively. Poultry meat meal combined with spray dried haem was better digested at 75%. These results are very encouraging for the use of animal by products in diets for marine flat fish species. These protein digestibility values are also displayed in figure 3.

The relative essential amino acid digestibility (DC) data for the ingredients for turbot are displayed in figure 4. Clearly despite the overall similarity to the protein digestibility coefficients measured for turbot, there are a number of important differences obtained for specific amino acids within the test ingredient products.

For turbot, it was noticed that all essential amino acid (EAA) digestibility coefficients were slightly lower in value compared to those reported previously for sea bass. Figure 4 shows these values for all of the EAA's and difference can be discerned.

Very low DC values were measured for cysteine (24.98%; 8.24%) for both steam hydrolysed and enzyme treated feathermeal respectively whilst higher values were obtained for methionine with a better result for enzyme treated product (69.34% v 74.90%). This was similar to the trends obtained for sea bass with this product. Generally, enzyme treatment of feathermeal was not effective to raise the amino acid digestibility for turbot. Spray dried haem was fairly good and compared favourably with fishmeal (80-90% for most EAA's) with respect to lysine, phenyl alanine, arginine, cysteine, leucine, threonine and tryptophan, but was quite poor with respect to methionine and isoleucine (51.25% & 44.90%). For turbot, poultry meat meal was not as well assimilated in terms of specific essential amino acids compared to its performance with sea bass. Histidine for instance, was only apparently 45% digested and cysteine at 43% availability for turbot. All combinations of protein blends produced EAA digestibility coefficients that reflected each ingredient and the ratios resulted in reduced overall EAA DC values for turbot. It would appear that the poultry meat meal: spray dried haem blend was the superior combination with respect to EAA availability (61-83.35%) with cysteine showing the lowest DC at 38.84%.

Energy digestibility coefficients for complete diets showed relatively uniform coefficients with some variations obviously reflecting the animal protein ingredients. Coefficients range from 53% to 81%. The best values were for poultry meat meal, poultry meat meal spray dried haem blend, and the spray dried haem (80%). The enzyme feathermeal/spray dried haem was 71% digested and both feathermeals at 77%. The lowest value (53%) was for the feathermeal spray dried haem blend. Finally the lipid digestibility coefficients ranged between 57% and 80% (the reference fishmeal diet being the highest value at 80%). The lower value (57%) was produced by the enzyme feathermeal diet, but interesting the higher value was observed for hydrolysed feathermeal. Poultry meat meal, spray dried haem, poultry meat meal spray dried haem blend and feathermeal spray dried haem blend all had similar values at 70%. Of interest again was that the enzyme feathermeal/spray dried haem produced a better result for lipid digestibility (63%).

Discussion

The broad strategic rationale for evaluating alternative proteins in fish diet formulations were eloquently described by Allan and Rowland (1998) with respect to the Australian silver perch. The techniques and methodological approaches described by these workers for the assessment of novel proteins and feed ingredients have been adopted world-wide and forms the basis of fish nutrition research protocols. In our studies with marine fish (sea bass and turbot), classical experimental designs for the determination of digestibility coefficients for a selection of terrestrial animal derived proteins were adopted.

The experimental trials demonstrated that specific classes of animal by-products were highly digested for both sea bass and turbot juvenile fish within the scope of a limited feeding trial for evaluation of digestibility profiles of key nutrient components. The test ingredients were incorporated into a matrix that closely resembled typical commercial diets for these species and were fed according to standard practices as proposed in the scientific literature. These were similar to the approach used by Lupatsch *et al* (1997) working with Gilthead sea bream, *Sparus aurata*. This protocol has been adopted by Bureau et al (1999; 2000) for work to evaluate the digestibility of animal proteins for rainbow trout.

Turbot and sea bass are very different in many respects and the former has a much shorter gastrointestinal length and tends to have a more defined feeding frequency and meal intake preferring larger pellet sizes. Sea bass in contrast may be expected to consume proportionally smaller meals but both fish are regarded as carnivores dependent on high protein and energy rich diets respectively. Digestibility experiments are the classical approach to appraising potential feed ingredients for fish prior to full balanced diet formulations using long term feeding studies where growth and other criteria of performance may be made. Investigations by (McGoogan & Reigh, 1996; Lee, 2002; Laining, 2003) on a number of important fish species including red drum, *Sciaenops ocellatus*, rockfish, *Sebastes schlegeli* and humpback grouper, *Cromileptes altivelis* has revealed the characteristics and feasibility of novel protein sources including several varieties of animal derived proteins. These workers employed the fixed ingredient level concept in mixed diets where the test ingredient is substituted for a basal or reference diet also fed to the species in question. This technique is the basis for the Guelph method and

the ratio of test ingredient to reference diet serves to allow the calculation of nutrient digestibility after collection of faeces from each treatment. There have been many criticisms of the method by some researchers but the focal point of concerns is usually directed towards the choice of inert dietary marker or faecal collection technique. Others have even questioned the validity of the calculation itself and suggested modifications to the equation of digestibility suited to the technique (Forster 1999).

For salmonid fish, the anatomical features is such, that the manual stripping method is often used since this is ideal for rapid removal of faecal material and is not prone to the leaching losses that can result in the over- estimate of the digestibility coefficients for each nutrient class.

In both sea bass and turbot however, it is not possible to strip fish of faeces and so the material was obtained by the use of the Guelph digestibility tank and daily collection of naturally voided faeces in traps. This approach may result in higher values for calculated digestibility coefficients but in the case of the study with sea bass and turbot, the data would nonetheless be expected to be of relative order for the absorption of crude protein, amino acids, lipid and energy.

The palatability of the diets were found to be highly acceptable for sea bass and turbot in the current study, although some problems were encountered for the diet containing a blended source of spray dried haem and steam treated feathermeal proteins for the sea bass investigation. This diet was found to be potentially unstable and the high levels of iron in the haem appeared to cause a progressive rancidity during storage. All, remaining diets were consumed by fish resulting in copious amounts of faeces for analysis.

The results were consistent with the information provided by previous workers and confirmed the very high availability of the animal proteins for fish.

Typically, a low temperature LT fish meal proved superior in both fish species in terms of overall protein and amino acid digestibility with values only slightly inferior for turbot compared to sea bass. The advantages and merit of fishmeal for aquafeeds has been extensively promoted and Pike et al. (1990) reviewed the beneficial role of fishmeal in diets for salmonid fish.

Processing of any raw material is the main criteria governing quality and scope for inclusion in balanced diets. It is imperative that the protein is highly available and hence

properly digested within the gastrointestinal tract of fish. Bureau et al (1999) provided valuable information on the apparent digestibility of rendered animal protein ingredients for rainbow trout and included feathermeals in the study.

The processing of feathermeal with an enzymatic treatment did not yield any significant benefit over the standard steam hydrolysed method which is in contrast to previous (unpublished data) for rainbow trout by the current investigators. In a previous study by Pfeffer and Henrichfreise (1994) these investigators reported that feathermeal was an acceptable partial protein alternative to fishmeal in diets for trout with a protein digestibility of 67% for the standard steam hydrolysed product evaluated. This value compared favourably with the data obtained for turbot but higher values of protein digestibility was obtained for sea bass.

Spray dried haem as a single ingredient was very well digested by both species with respect to protein and its amino acids. Various blood meals have proven effective for other fish species as well, and Davies et al (1991) were able to confirm this in experiments with juvenile tilapia *Oreochromis mossambicus.*

Poultry meat meal has been favourably reported for many fish species (Fowler, 1990:1991; El-Sayed, 1999; Abdel-Warith et al., 2000) and the present studies support the potential of this by-product in aquafeeds for bass and turbot with very promising results. Indeed, poultry meat meals are probably the most effective of the animal proteins currently available from both a nutritional standpoint and from the bioethical considerations.

Nengas et al (1999) were able to show the effective use of a standard grade poultry meat meal for Gilthead sea bream, *Sparus aurata*. These workers reported excellent growth and digestibility in balanced diet formulations for juvenile production class of this important marine fish species widely cultured in the Mediterranean region.

Interestingly, the digestibility data indicated the possibility of significant interactions between nutrients and this was apparent for the digestibility of lipid and energy for the diet fed to sea bass containing a blend of steam hydrolysed feathermeal and spray dried haem protein concentrate. Although less of an effect for turbot, it does point to the inherent risk of mixing various ingredients and creating an antagonistic effect.

Combining feathermeal and blood meal for both species was clearly not advantageous and requires more testing to elucidate the physiological causes of this result.

The protein digestibility values reflect the specific amino acid availability profile of the ingredients evaluated and ultimately affect the efficiency of protein utilisation and growth performance of the fish. These showed that the overall protein digestibility index masks important differences between animal protein sources with respect to each individual amino acid. There were marked variations and many similarities for specific EAA coefficients between diets and fish species evaluated in the trials. The results for the sulphur amino acids cysteine and methionine were particularly interesting for feathermeal due to the known poor protein digestibility characteristics of this material for most fish. Although the availability of cysteine was poor for both turbot and sea bass the combined cysteine and methionine digestibility was reasonably good for feathermeal protein concentrates. Poultry meat meal was consistently superior in general performance for both fish species and would be considered to be high quality protein source for fish diets.

This study is amongst very few that have explored the detailed digestibility coefficient patterns for all essential amino acids in animal protein sources for marine fish. Other workers (Anderson et al, 1992) have provided data for mainly salmonid fish such as and salmon, and some limited information for other species such as striped bass, Murray cod, eel is available (Small et al, 1999; De Silva et al, 2000). Recently Portz and Cyrino (2004) reported the digestibility of nutrients and amino acids of different protein sources in practical diets by largemouth bass *Micropterus salmoides*. Data for this carnivorous fish species included essential and non-essential amino acid availability for plant and animal protein sources in practical diet formulations. Feathermeal and poultry by - product meals were included in this study and low ADC values for certain amino acids were described. It was stated that this was mainly due to the quality and processing of the feedstuffs and in particular the method of extraction of lipids.

The current investigation with sea bass and turbot has yielded valuable numerical coefficients for apparent digestibility for EAA's regarded as of prime importance in generating balanced diet formulations for fish. One area that has been highlighted is the important differences in the performance of sea bass and turbot for the diets ingredients tested. This demonstrates that caution must be made between extrapolating results fro one

species to another and this has been a common aspect of salmon and rainbow trout work previously.

Future experiments need to explore the absorption and possible interactions between essential amino acids from different animal proteins and compare the plasma levels with those of fish receiving fishmeal as a reference protein. The relative retention of EAA's should also be compared with the reference fishmeal based diet to provide more information on the Biological Value (BV) of novel animal proteins of the type used in the current investigations.

In general the present digestibility trials were able to provide valuable preliminary data necessary for the optimum inclusion of animal proteins in diets for sea bass and turbot. The coefficients of digestibility will thus allow more accurate substitution of ingredients on an equivalent protein and amino acid basis and also with respect to balancing the energy level of the diets. The next phase of these experiments will decide the best sources of mainly poultry by-products and blood meals for further testing and trials will focus on growth and feed utilisation of complete diet formulations. An important objective will be to establish the effects of fish meal substitution on general health and quality of fish with special attention to haematological and immune parameters. This will feature in planned studies for Gilthead sea bream in particular.

This will allow more accurate predictions for the potential use of selected animal protein sources and combinations for sea bass, turbot and also Gilthead sea bream diets. Such investigations will help provide a comprehensive nutritional assessment strategy for these important Mediterranean marine fish species.

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Table 1 Sea bass diet formulation (g/kg) & nutrient analysis (%)

Ingredients	Ref	SHF	ETF	PMM	SDH	SHF & SDH	PMM & SDH	ETF & SDH
Fishmeal Norwegian (LT-94)	600	200	200	200	200	200	200	200
Marine fish oil	100	120	120	100	120	120	100	100
Corn starch	212	201	201	212	201	201	214	201
Dextrin	68	59	59	68	59	59	66	59
Vitamins	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Minerals	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Chromic oxide	5	5	5	5	5	5	5	5
Steam hydrolysed feathermeal	0	400	0	0	0	300	0	0
Poultry meat meal	0	0	0	400	0	0	300	0
Spray dried haem	0	0	0	0	400	100	100	100
Enzyme treated feathermeal	0	0	400	0	0	0	0	300
Total	1000	1000	1000	1000	1000	1000	1000	1000
Moisture	9.3	9.6	8.5	7.8	8.8	9.3	9.9	7.8
Crude Protein	41.54	45.06	42.12	39.48	47.75	43.81	40.38	42.56
Lipid	12.82	13.81	14.86	14.08	13.04	13.44	13.46	14.61
Ash	9.20	4.73	5.68	8.34	5.05	4.73	7.36	5.18
Energy	22.06	23.13	23.05	21.87	22.11	23.01	21.97	22.85

Table 2 Turbot diet formulation (g/kg) & nutrient analysis (%)

Ingredients	Ref	SHF	ETF	PMM	SDH	SHF & SDH	PMM & SDH	ETF & SDH
Fishmeal Norwegian (LT-94)	650	250	250	250	250	250	250	250
Marine fish oil	102	132	132	114	150	137	123	123
Corn starch/Dextrin	228	298	298	316	280	293	307	307
Vitamins/Mineral premix	15	15	15	15	15	15	15	15
Chromic oxide	5	5	5	5	5	5	5	5
Steam hydrolysed feathermeal	0	300	0	0	0	225	0	0
Poultry meat meal	0	0	0	300	0	0	225	0
Spray dried haem	0	0	0	0	300	75	75	75
Enzyme treated feathermeal	0	0	300	0	0	0	0	225
Total	1000	1000	1000	1000	1000	1000	1000	1000
Moisture	6.44	6.39	6.47	6.44	6.20	5.11	9.33	8.23
Crude Protein	46.35	45.63	44.75	46.76	46.32	47.87	42.82	44.84
Lipid	19.10	20.96	21.80	19.23	20.50	21.51	21.19	21.13
Ash	9.98	5.46	5.88	7.48	5.56	5.24	7.70	5.17
Energy	21.50	21.97	22.01	21.28	22.54	21.76	21.85	21.21

Table 3 Sea bass digestibility (diets & ingredients ± SEM, n=3)

Parameter	Ref	SHF	ETF	PMM	SDH	SHF & SDH	PMM & SDH	ETF & SDH
Dry matter	77.95 ± 1.16	67.51 ± 1.16	69.06 ± 0.19	70.11 ± 2.23	73.21± 1.71	47.21 ± 3.17	73.40 ± 1.05	64.23 ±
Protein (diet)	93.49 ± 1.44	78.93 ± 2.51	79.15 ± 2.04	89.53 ± 0.69	92.11 ± 1.02	72.63 ± 2.55	90.83 ± 0.86	74.10 ± 5.14
Protein (ingredient)	93.49 ± 1.44	75.46 ± 6.98	74.71 ± 3.73	83.22 ± 5.47	88.54 ± 1.46	65.51 ± 11.73	82.20 ± 7.06	71.07 ± 11.74
Energy (diet)	87.94 ± 0.88	75.82 ± 1.26	77.32 ± 1.00	81.37 ± 1.53	79.56 ± 0.88	53.00 ± 2.60	81.63 ± 0.84	71.28 ± 2.47
Lipid (Diet)	90.06 ± 1.02	71.91 ± 1.63	71.93 ± 4.69	82.79 ± 2.04	68.56 ± 0.38	0< ± n/a	76.72 ± 1.07	61.24 ± 5.65

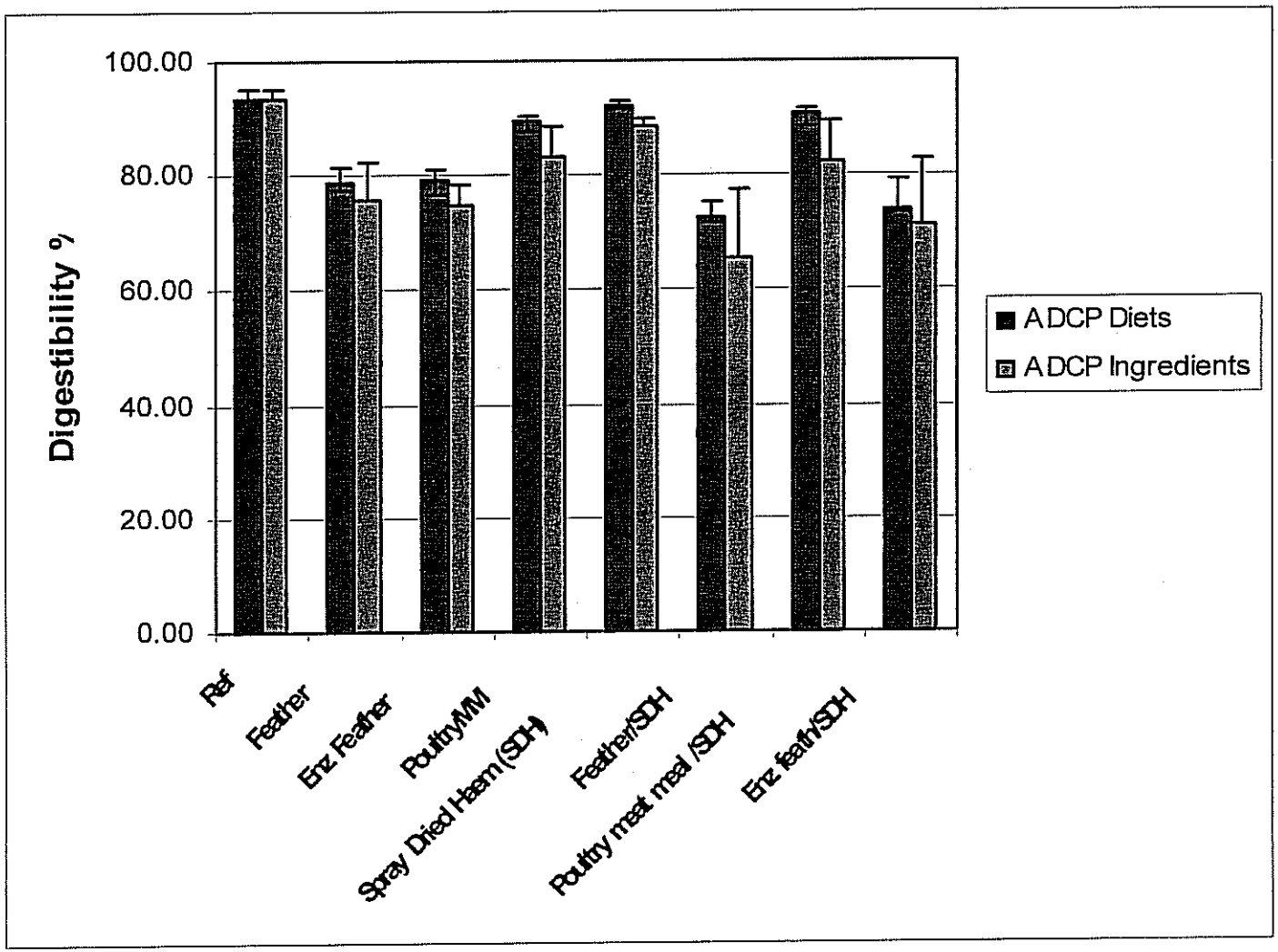

Fig.1 Sea bass protein digestibility, test diets and ingredients

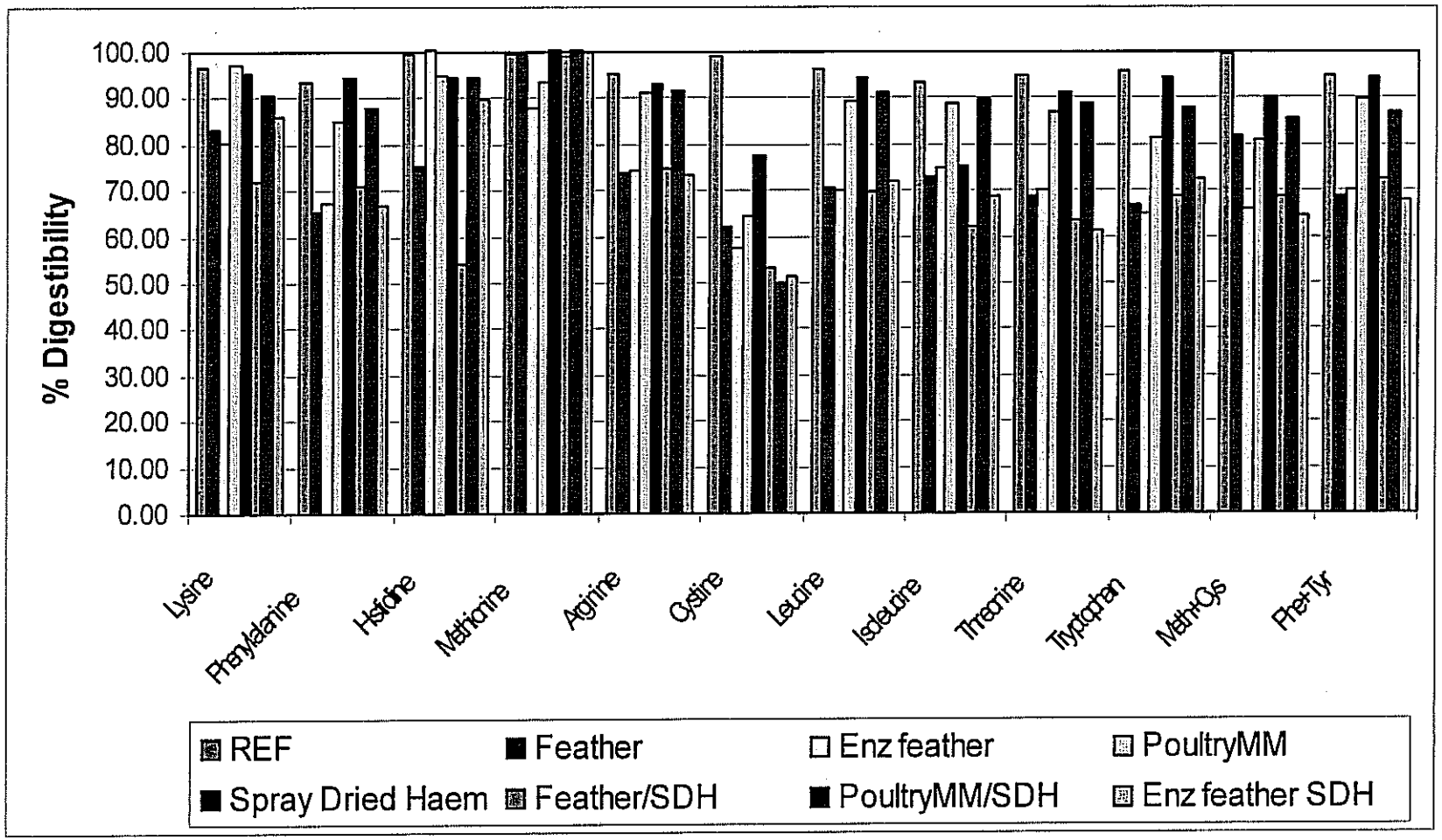

Fig. 2 sea bass essential amino acid digestibility (ingredients)

Table 4 Turbot digestibility (diets & ingredients ± SEM n=3)

Parameter	Ref	SHF	ETF	PMM	SDH	SHF & SDH	PMM & SDH	ETF & SDH
Dry matter	66.12 ± 3.45	51.18 ± 2.53	43.60 ± 4.38	53.49 ± 2.51	54.90 ± 1.53	49.96 ± 2.61	49.28 ± 3.48	42.63 ± 2.89
Protein (diet)	84.93 ± 2.26	68.98 ± 5.96	63.86 ± 3.26	82.58 ± 4.53	80.42 ± 1.82	72.39 ± 3.92	75.60 ± 3.83	71.30 ± 4.14
Protein (ingredient)	84.93 ± 2.26	63.02 ± 11.93	58.42 ± 2.92	66.00 ± 15.16	79.40 ± 3.90	66.25 ± 8.23	71.36 ± 5.75	67.27 ± 2.66
Energy (diet)	74.46 ± 3.58	57.28 ± 2.61	48.74 ± 5.11	61.15 ± 3.03	62.52 ± 1.56	55.29 ± 2.98	57.22 ± 3.80	49.05 ± 3.54
Lipid (Diet)	81.05 ± 3.29	66.15 ± 3.46	54.51 ± 5.96	71.48 ± 1.85	69.04 ± 3.85	68.99 ± 2.73	67.25 ± 4.11	61.93 ± 3.22

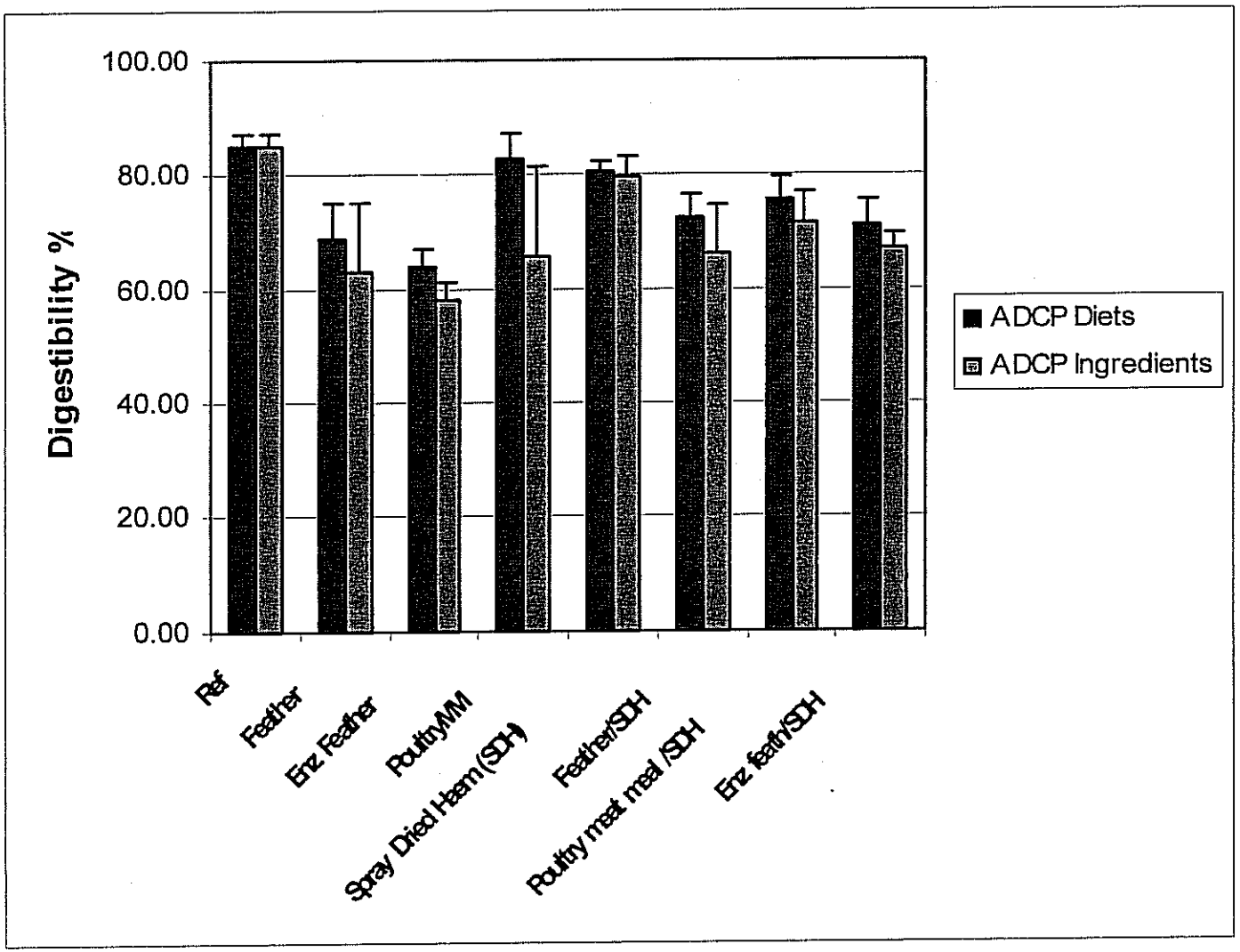

Fig. 3 turbot protein digestibility, diets and ingredients

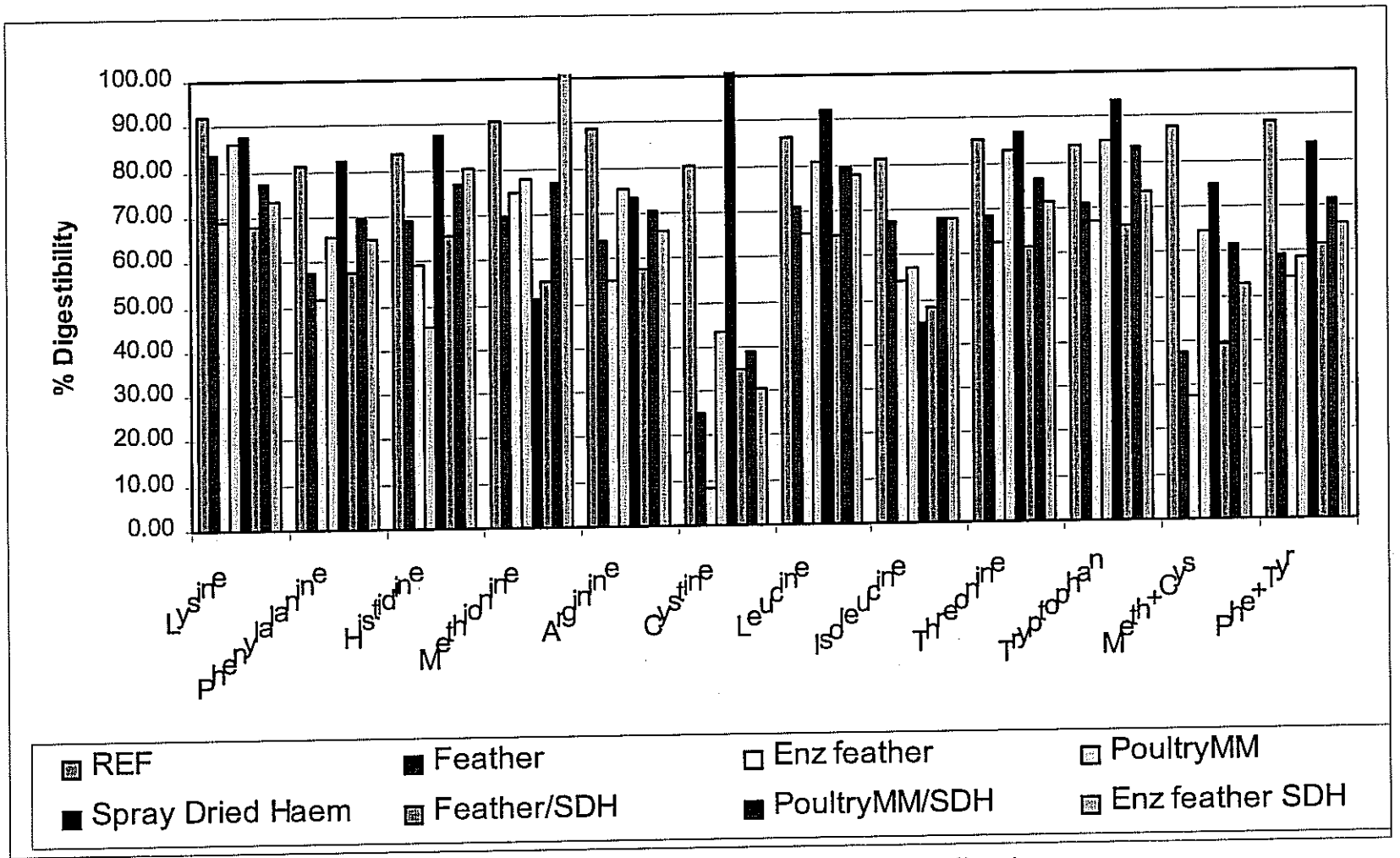

Fig. 4 turbot essential amino acid digestibility (ingredients)