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Assessment of Apparent Digestibility Coefficients (ADCs %) of some Animal Protein Sources by Gilthead Sea bream (*Sparus aurata*)

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Abstract

The present study was conducted to evaluate the Apparent Digestibility Coefficient (ADC %) of five animal protein sources (squid meal, imported fish meal 62% CP, sardine meal, shrimp meal and meat meal) by Gilthead Sea bream, *Sparus aurata* (with an average body weight of 100 g ± SD). Results revealed that ADC% ranged from 62.24% to 84.36% for ingredient protein content and from 71.68% to 79.79% for lipid. Fish meal was recorded the highest ADC% of protein (84.36%) among different animal protein sources, followed in decreasing order by squid meal (81.1%) and sardine meal (78.0%) compared to the lowest ADC% of protein which recorded to shrimp meal (62.24%) and meat meal (65.32%). All single ingredients tested in the present study showed the highest ADC% of fat content except shrimp meal (71.68). In addition, squid meal recoded the higher digestible crude protein (DCP): digestible energy (DE) ratio, while the lowest was observed in meat meal. In conclusion, diets for *Sparus aurata* could be formulated on the basis of digestibility of individual ingredients and squid meal protein could be recommended to use as alternative protein source in *S.aurata* diets.

Keywords: Protein, digestibility, coefficient, squid meal, shrimp meal, sardine meal, meat meal.

1. Introduction

Gilthead sea bream is one of the most important marine finfish species cultured in the Mediterranean region and its production is still in rapid expansion (Basurco and Abellán, 1999). It is considered one of the euryhaline and eurythermal fishes, carnivorous and accessorily herbivorous (Bauchot and Hureau, 1990). The species inhabits sea grass beds and sandy bottoms as well as the surf zone, shows high adaptability to intensive culture conditions, where balanced complete diet plays an important role in providing nutritional requirements.

However, aquaculture feeds are amongst the most expensive animal feeds account for half of the total cost of aquaculture production, with protein being the most expensive component (Southgate, 2003 and Lunger *et al.*, 2007). Accordingly, due to their high nutritional content, marine protein meals such as fish meal, squid meal, meat meal and shrimp meal have long been the main protein sources used in feeds for most aquaculture species, where marine meals are generally incorporated into feeds at levels between 30% and 60% (Ogunkoya *et al*., 2006). These ingredients have low of zero anti-nutritional factors, they have little or no carbohydrate and are widely available.

As some ingredients cannot be fed as sole feed, knowledge of digestibility of single feeds must be based on evaluation of digestibility studies in which test ingredients have been blended with reference diets of known digestibility. By using a reference ingredient care must be taken that the inclusion level of the nutrient in question is high enough to make the interpretation reproducible.

Research over many years has demonstrated that rendered feeds are well digested and utilized for aquaculture species (Allan *et al*., 2000; Booth *et al*., 2005; Bureau, 2006; Davies *et al*., 1989, 1993; Stone *et al*., 2000; Sugiura *et al*., 1998; Watanabe *et al*., 1993; Williams *et al*., 1998).

Historically, fish meal has been considered the most acquisitive feed ingredient for most fish species in spite of being expensive because of its palatability and ability to support rapid growth of aquaculture. It was estimated, in 2006, that 68.2 and 88.5% of global production of fishmeal and fish oil, respectively were used in aquafeeds (Tacon, 2008), and even assuming continuing long term sustainable production of fishmeal and fish oil, for aquaculture continuous growth, additional protein and energy sources are needed.

For aquatic animals, apparent digestibility is determined by an indirect method using the difference in ratios of ingested and egested marker and nutrient,

where the quantity of a nutrient consumed is compared with that in feces at the end of the digestive process.

Because the dietary nutrients requirement is the basis for their inclusion levels in feed formulation, it would be important to know its digestibility coefficients, which depends primarily on its chemical composition and the digestive capabilities of the species to which it is fed (McGoogan and Reigh 1996).

The main objective of the present study is to investigate the apparent digestibility coefficients of single ingredient from animal protein sources by Gilthead Sea bream (*Sparus aurata*).

2. Material and Methods

This study was conducted to determine ADCs of crude protein (CP), fat (EE) and gross energy (GE) for squid meal, fish meal (imported, 62% CP), sardine meal, shrimp meal and meat meal as single protein sources of tested diets fed to Gilthead sea bream (*Sparus aurata*).

2.1. Experimental Fish

Experimental fish were selected from Damietta Governorate Farm, Al-Rattama, acclimated to fine experimental treatments for seven days. Five groups, 3 fish per treatment, of *S.aurata*, with an average of 100 g ± SD body weight/ fish, were collected and distributed in triplicate per group. Fish in each treatment were starved for 24 hours before starting the digestibility trial.

2.2. Experimental Aquaria

Thirty glass aquariums, 6 mm thickness, 100 cm in length, 40 cm in width and 30 cm in height, were used in the present experiment. Water volume in each aquarium was adjusted at 100 liter of filtrated sea water. Fifteen aquariums were used for the purpose of fish feeding where fish were held for two hours, and then fish were transferred to the another fifteen aquariums for collection of excreted faeces. Each treatment was in triplicate. About one third of the water volume was replaced every morning with a new volume of fresh sea water, before the first feeding. Culture aquaria were aerated using air pumps.

2.3 Animal Protein Sources and Diets Preparation

Five animal protein sources were investigated as single ingredient diet: squid meal (*Stoloteuthis leucoptera*), fishmeal (imported, 62% CP), sardine meal (*Sardina pilchardus*), shrimp meal (*Penaeus semisilcatus*) and meat meal.

Feed ingredients were dried at 70°C and grinded in hammer mills. Then 1% carboxy methyle cilliobiase (CMC) was added as a binder for each diet and 0.5% chromic oxide (Cr_2O_3) was added as inert indicator. The different experimental diets were well homogenized and then 25% water was added to the dry meals. Homogenized mixture was pelletized through a meat mincer and stored at -4°C until use.

Before the beginning of the experiment, fish have been fed test diets for seven days acclimation period in all treatments once daily (8.00 am) at a rate of 1% of its live body weights. The concentrations of nutrients (protein and lipid) and the inert indicator (Cr_2O_3) were determined in both the food and the faeces. The apparent digestibility coefficient (ADC %) of each nutrient was calculated according to the following formula:

$$ADC\% = 100 - \{ 100 \times \%Cr_2O_3 \text{ in diet} / \%Cr_2O_3 \text{ in faeces} \times \% \text{ nutrient in faeces} / \% \text{ nutrient in diet} \}$$

Table 1. Formulation of the experimental diets (% On DM basis).

Ingredient	Single ingredient diet				
	Squid meal	Fishmeal	Sardine meal	Shrimp meal	Meat meal
Squid meal	98.50	---	---	---	---
Fishmeal	---	98.50	---	---	---
Sardine meal	---	---	98.50	---	---
Shrimp meal	---	---	---	98.50	---
Meat meal	---	---	---	---	98.50
CMC	1.00	1.00	1.00	1.00	1.00
Cr_2O_3	0.50	0.50	0.50	0.50	0.50
Total	100	100	100	100	100

CMC: Carboxy Methyl Cilliobiase
Cr_2O_3: Chromic oxide

2.4. Faeces Collection and Preparation of samples

Faeces were collected once it was excreted and sedimented to prevent nutrients leaching and dried at 70°C, then it was grinded in hammer mills and stored in desiccators for subsequent analyses. Experimental sample diets and faeces were analyzed for chromic oxide content according to Bolin *et al.* (1952).

This study was continued for 3 weeks. One gram of pooled dried faeces composite sample of each treatment was weighed and ashed at 600°C for 1.5 hr. When samples had been cooled, 3 ml of phosphoric acid–manganese sulphate solution (30 ml of 10%, w/v, $MnSO_4.4H_2O$ solution in one liter of 85% phosphoric acid) and 4 ml of 4.5%, w/v, potassium bromate solution were added. Then samples were covered with watch - glasses and digested on a previously heated hot plate until effervescence ceased and a purple color appeared (about 5-7 minutes). Samples were cooled, diluted with water and washed completely into a 200 ml volumetric flask. In the next step, 25 ml of calcium chloride solution containing 4000 ppm of calcium was added, made to volume with water and mixed throughly. The samples were stood overnight to settle suspended material and then filtered. Blank was prepared using the same proceed and all samples were examined for chromium concentrations.

2.5. Analytical Methods

At the end of the trial, the samples of diets and faeces were analyzed for proximate chemical analysis. Contents of protein were determined using Kjeldahl method, fat by ether extract method, Soxhlet extraction, moisture by oven drying at 105°C for 24 h, ash (burn oven) according to procedures of Official Analytical Chemists AOAC (1985) and energy content were calculated using the values of 5.65, 9.45, and 4.11 kcal/g for protein, lipid, and carbohydrates, respectively (NRC, 1993).

2.6. Statistical Analysis

Data were statistically analyzed by ANOVA using completely randomized design according to Steel and Torrie (1980). LSD range test procedure was used to compare differences between treatment means when significant F values observed using MSTAT-C (1994) software package (Ver. 2.11, 1994).

3. Results and Discussion

Table (2) illustrates the chemical analysis of the tested individual ingredients that used in the present study. The data observed that squid meal was the higher in crude protein content (72.82%), gross energy (513.12 kcal/100g DM) and protein: energy ratio (141.70 mg CP/kcal GE) compared to other protein sources, while ash content recorded the lowest value (7.83%). Ether extract (EE) content in sardine meal (11.65%) was the highest value compared to other protein sources followed by fishmeal (7.64%) and squid meal (5.89%) in decreasing order. Lowest EE content was recorded in shrimp meal (3.77%).

The data in Table (3) shows the proximate chemical composition of excreted faeces for different experimental animal protein sources. Data indicated that CP% in the faeces of fish fed meat meal was significantly ($P<0.01$) higher (34.0%) than fish fed other animal protein sources, while the lowest CP% was recorded in the faeces of fish fed squid meal (24.76%). The highest value of EE% was recorded with fish fed sardine meal (5.72%) and the lowest values were observed for fish fed shrimp meal (1.82%). The values of ash content were significantly ($P<0.01$) higher in all treatments except for fish fed squid meal (14.88%). Moreover, fish fed squid meal excreted more GE (396.3 kcal/ 100 g DM), compared to other treatments.

Table (4) presents the apparent digestibility coefficient (ADC%) of protein experimental animal protein sources fed to *S. aurata.* The highest values of ADC% for crude protein was recorded for fish meal followed by squid meal, sardine meal, meat meal and shrimp meal in decreasing order 84.6%, 81.2%, 78.1%, 65.2% and 62.4%. It was observed that differences were not significant ($P<0.01$) between all animal protein sources in fat ADC% except for shrimp meal. The highest values for DE were found in sardine meal (338.2 kcal/100g) and squid meal (333.1 kcal/100g), while the lowest value was observed for fish fed shrimp meal (141.6 k cal/100g).

The highest significant difference ($P<0.01$) for DCP: DE was recorded for fish fed squid meal (144.3 mg/ kcal) while, meat meal recorded the lowest value (65.74 mg /kcal).

Evaluation of apparent digestibility coefficient for dietary ingredients in fish nutrition is contribute better to assess their potential nutritional value and nutrients availability through short term digestibility trials, which help in optimum diet formulation. In this regard, chemical composition and quality of meals differ considerably depending on raw materials and processing methods (Dong *et al*., 1993). Energy content of the feed is considered to be the main factor controlling feed consumption in finfish (Jobling and Wandsvik, 1983; Kaushik and Luquet, 1984; Kaushik and Oliva Teles, 1985; Boujarda and Medale, 1994; Paspatis and Boujarda, 1996; Boujarda *et al.*, 2004). In the present study, the data of proximate chemical composition revealed that squid meal is the richest animal protein source in crude protein and gross energy among tested animal protein sources. Fish meal, imported 62% CP, was the second tested animal protein source in CP%, EE%, ash content and the lowest CF% value. Sardine meal was found to be high in EE% and low in CF%, meanwhile shrimp meal was found to be higher in CF% and ash content.

Table 2. Proximate Chemical analysis of the tested animal protein sources.

Single Protein Diet	% on DM basis					GE (kcal/100g)	P:E (mg/kcal/GE)
	DM %	CP %	EE %	CF %	Ash %		
Squid meal	84.66 ± 0.20	72.82 ± 0.11	5.89 ± 0.06	2.07 ± 0.07	7.83 ± 0.02	513.12 ± 0.01	141.7± 0.07
Fish meal	91.00 ± 0.13	62.60 ± 0.10	7.64 ± 0.02	0.67 ± 0.08	18.24 ± 0.11	470.48 ± 0.06	133.1± 0.20
Sardine meal	93.27 ± 0.30	59.30 ± 0.18	11.65 ± 0.02	1.30 ± 0.08	17.60 ± 0.18	486.85 ± 0.13	121.8 ± 0.05
Shrimp meal	86.34 ± 0.38	51.00 ± 0.48	3.77 ± 0.06	10.46 ± 0.03	22.60 ± 0.02	373.80 ±0.10	136.4 ± 0.07
Meat meal	91.0 ± 0.12	50.00 ± 0.11	4.40 ± 0.03	3.10 ± 0.08	17.00 ± 0.03	428.89± 0.04	116.6± 0.21
L.S.D. (P< 0.01)	0.639	0.629	0.116	0.183	0.246	0.200	0.357

Means in the same column with the same letters are not significantly different (P< 0.01).
DM: Dry matter CP: Crude protein EE: Ether extract CF: Crude fiber
NFE: Nitrogen free extract GE: Growth Energy P: E: Protein/energy.

Table 3. Proximate Chemical analysis of excreted faeces of sea bream.

Single Protein Diet	% on DM basis				GE (kj/100g)	P:E (mg/kj/GE)
	CP %	EE %	CF %	Ash %		
Squid meal	24.76^{e} ± 0.02	2.84^{c} ± 0.03	1.66^{c} ± 0.02	14.88^{e} ± 0.03	396.3^{a} ± 0.04	62.48^{e} ± 0.02
Fish meal	27.64^{d} ± 0.03	3.61^{b} ± 0.01	0.50^{e} ± 0.03	27.12^{b} ± 0.03	359.3^{a} ± 0.01	76.93^{d} ± 0.02
Sardine meal	29.66^{c} ± 0.03	5.72^{a} ± 0.01	0.85^{d} ± 0.03	25.82^{c} ± 0.03	377.6^{b} ± 0.14	78.55^{c} ± 0.02
Shrimp meal	32.60^{b} ± 0.05	1.82^{e} ± 0.01	6.66^{a} ± 0.02	29.61^{a} ± 0.03	321.9^{e} ± 0.03	101.27^{a} ± 0.01
Meat meal	34.00^{a} ± 0.06	1.97^{d} ± 0.02	1.86^{b} ± 0.02	25.15^{d} ± 0.04	360.3^{c} ± 0.02	94.37^{b} ± 0.01
L.S.D. (P< 0.01)	0.116	0.026	0.082	0.082	0.183	0.026

Means in the same column with the same letters are not significantly different (P< 0.01).
CP: Crude protein **EE:** Ether extract **CF:** Crude fiber
NFE: Nitrogen free extract **GE:** Growth Energy **P: E:** Protein/energy

Table 4. Digestibility coefficient of protein, fat and igestible protein: digestible energy ratio for experimental diets, by *Sparus aurata*.

Single Protein Diet	ADC % (protein)	ADC % (Fat)	DE (kcal $100g^{-1}$)	DCP:DE (mgCP kcal/DE^{-1})
Squid meal	81.2^{b} ± 0.06	78.0^{a} ± 2.02	333.1^{b} ± 0.14	144.3^{a} ± 0.11
Fish meal	84.6^{a} ± 0.11	79.7^{a} ± 1.73	316.3^{c} ± 0.18	110.5^{c} ± 0.11
Sardine meal	78.1^{c} ± 0.63	78.4^{a} ± 1.60	338.2^{a} ± 0.09	87.64^{d} ± 0.07
Shrimp meal	62.4^{e} ± 0.05	71.1^{b} ± 0.47	141.6^{e} ± 0.19	130.0^{b} ± 0.12
Meat meal	65.2^{d} ± 0.07	77.2^{a} ± 1.03	243.4^{d} ± 0.11	65.74^{e} ± 0.10
L.S.D. (P< 0.01)	0.088	3.872	0.384	0.271

Means in the same column with the same letters are insignificantly different (P< 0.01).
Cr_2O_3 in diet = 0.5%
ADC %: Apparent digestibility coefficient.
GE_f: Gross energy of faeces.
DP: DE: Digestible protein: digestible energy.

3.1. Protein digestibility

In the present study, high protein content (24.76%-34.0%) was observed in the faeces of all treatments, however high protein loss in fish faeces may be due to that the amino acid sequence of the protein is not easily attacked by the proteases (Alarcon *et al.*, 1997). On the other hand, all fishes were fed in the present study once daily at 1% of live body weights, which indicates certainly to feed deprivation and, in turn, in such circumstances protein becomes an important energy source (Eroldoğan *et al.*, 2008).

Values of the apparent digestibility coefficient (ADC %) of crude protein revealed that fish meal protein was highly digested by *S.aurata* (84.6%), followed by squid meal and sardine meal proteins (81.2 and 78.1%, respectively). Pike *et al*. (1990) concluded that digestibility of fish meal may be improved by

employing low temperature in the drying process. On the other hand, meat meal and shrimp meal crude proteins (CP) had lower digested by *S.aurata* (65.32 and 62.24%, respectively). Axelrod (1996) revealed that squid meal is considered a high digestible protein source, which provides a full range of amino acids and various kinds of vitamins, minerals for fish and also 1.0-1.5% of cholesterol that is suitable for fry and young marine fish.

The highest ADC% of crude protein found in fish meal and the lowest value in shrimp meal in the present trial, may be attributed to low fiber content in fish meal (0.67%) and high fiber content in shrimp meal (10.46%). Kirchgessner *et al*. (1986) indicated that there was a negative correlation between protein digestibility coefficient and crude fiber content in animal protein sources. This negative correlation between ADC% of protein and CF content was explained by Hanley (1987) who suggested that the absorption of water by the fiber component of diets containing high levels of fiber resulted in a reduction in gut transit time and a consequent reduction of protein and energy digestibility. In this connection, Lupatsch (2004) found that protein digestibility of individual ingredient of fishmeal, meat meal and squid meal ranged from 80-88%, 78-79% and 88%, respectively.

3.2. Fat digestibility

The non significant differences (P>0.01) and highly digested fat by *S.aurata* in all treatments except for shrimp meal reflect as well the negative correlation between dietary crude fiber content and fat ADC%. In comparison, result of fat ADC% for fish meal in the present study (79.7%) is lower than those reported by Sugiura *et al*. (1998) for herring meal (94.9%) and menhaden meal (89.9%) for rainbow trout (*Oncorhynchus mykiss*), which interpreted by Nose (1967), Takeuchi *et al.* (1979) and Austreng *et al.* (1980) who illustrated that the composition of the fatty acids; saturation level and thus the melting point have a strong influence on fat digestibility, where ADC% of fat decreases with increasing number of carbon atoms in the fatty acid chains and increases with the number of double bonds.

3.3. Energy digestibility

The higher fat content of sardine meal (11.65%), in the present study, resulted in higher digestible energy (338.2kcal/ 100 g), while lowest fat content in shrimp meal (3.77%) resulted in minimizing its digestible energy (141.6 kcal/ 100 g). The previous results were in agreement with Watanabe *et al*. (1979) who found that increasing fat content in feed increased the digestion of total energy by rainbow trout and they interpreted that this increase in DE was due to an increase in protein digestion, carbohydrate digestion and the lipids, themselves, digestion.

Gilthead Sea bream, *S. aurata* as a carnivorous fish has a physiological limitation to utilize carbohydrates. Tumison *et al*. (1939) and Phillips *et al*. (1948) stated that digestible carbohydrates in trout feed should not exceed 12%, since a higher content causes an accumulation of glycogen in the liver, associated with severe physiological disturbances and sometimes death of fish. In addition, it is well known that fish, especially salmonids, are considered as diabetic (non-insulin- dependent diabetes) and cannot utilize large quantities of carbohydrates in their feed (NRC, 1993).

As for shrimp meal, it is not (as a whole body with the chitinous membrane) a suitable source of animal protein for feeding *S.aurata.* This could be explained according to the lower digestibility coefficient of crude protein and fat, lower digestible energy content, and higher contents of crude fiber %.

4. Conclusion

In conclusion, the present results revealed that squid meal, fish meal and sardine meal are highly digested by gilthead sea bream, *Sparus aurata* and recommended to be included in practical fish diets, as animal protein source. However, shrimp meal could be included in *Sparus aurata* practical diets for a lesser extent with some precautions because of its high content of crude fiber. Diets for *Sparus aurata* could be formulated on the basis of digestibility of individual ingredients and squid meal protein could be recommended to use as alternative protein source in *S. aurata* diets.

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