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Growth response and body composition of sharpsnout sea bream (*Diplodus puntazzo*) fed a high energy diet with different protein levels

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ABSTRACT

A study was undertaken to determine the effect of a high energy diet with two different protein levels on growth, feed efficiency and whole body composition of sharpsnout sea bream (*Diplodus puntazzo*). Two isoenergetic diets (24.1-24.7 MJ Kg^{-1} dry weight) with two different protein levels (46.7 and 52.5% dry weight) were fed to satiety to duplicate groups of 300 fish (initial body weight 27.7 ± 0.2 g) for 94 days. At the end of the experiment, the fish fed 52.5% protein showed a statistically higher ($P < 0.05$) daily intake rate (DIR) of feed. Feed conversion rate (FCR) was similar among groups. Whole body composition was similar among treatments while the high energy level of the diets significantly modified lipid and moisture content in comparison with fish at the beginning of the experiment. Protein efficiency ratio (PER) and gross protein efficiency (GPE) of fish fed 46.7% protein diet were statistically higher than those for the other diet. It may be concluded that the diet with a lower protein level has given better protein utilization and a protein sparing effect but tended to result in reduced weight gain and feed intake, when compared with diet containing higher protein levels.

Key words: Diplodus puntazzo, Sharpsnout sea bream, P/E ratio, High energy diet, Growth

RIASSUNTO

ACCRESCIMENTI E COMPOSIZIONE CORPOREA DEL SARAGO PIZZUTO (*DIPLODUS PUNTAZZO*) ALIMENTATO CON DIETE AD ALTO CONTENUTO ENERGETICO A DIVERSI LIVELLI PROTEICI

*La presente ricerca è stata condotta per determinare l'effetto che diete con due differenti livelli proteici e un alto contenuto energetico, esercitano sulla crescita, l'efficienza alimentare e la composizione corporea del sarago pizzuto (*Diplodus puntazzo*). Gli animali, divisi in vasche contenti 300 pesci ciascuna, (peso corporeo iniziale di 27,7 + 0,2 g) sono stati alimentati a sazietà per 94 giorni con due diete isoenergetiche (24,1-24,7 MJ Kg^{-1} peso secco) e due livelli proteici pari al 46,7 e al 52,5 % del peso secco. Alla fine dell'esperimento, la dieta contenente il 52,5 % di proteina, ha mostrato un consumo alimentare giornaliero statisticamente più alto (*P *< 0,05). L'indice di conversione alimentare è stato simile fra i gruppi. La composizione centesimale della carcassa si è mostrata simile fra i gruppi mentre l'alto livello lipidico delle diete ha significativamente modificato il contenuto di lipidi e di umidità rispetto ai pesci all'inizio dell'esperimento. Il grado di efficienza proteica e l'efficienza proteica totale dei pesci alimentati con la dieta contente il 46,7 % di proteina sono stati statisticamente più elevati di quelli ottenuti nel gruppo alimentato con la dieta contente il 52,5*

% di proteina. E' possibile concludere che la dieta con un più basso livello proteico ha dato una migliore utilizzazione proteica ma tende a ridurre l'incremento di peso e l'assunzione alimentare, quando paragonata con diete contenenti livelli proteici più elevati.

Parole chiave: Diplodus puntazzo, *Sarago pizzuto, Rapporto proteina/energia, Diete ad alto contenuto di energia, Accrescimento.*

Introduction

In the Mediterranean sea, the dramatic increase in production and the subsequent price drop of sea bass and sea bream during the last decade, has brought the need to find new fish species suitable for farming. Among candidate alternative species, fish belonging to the *Sparidae* family seem of particular interest and several attempts have already been made in order to compare their rearing requirements (Divanach *et al.*, 1993; Abellàn *et al.*, 1997, Company *et al.*, 1999a). Encouraging results (Orban *et al.*, 2000; Hernàndez *et al.*, 2001; Favaloro *et al.*, 2002) were obtained regarding sharpsnout sea bream (*Diplodus puntazzo*), although more data should be collected to better understand the real potential of this fish. As very little is known of nutritional requirements of cultured sharpsnout sea bream, the development of proper and cost-effective feeds appears essential to improve the profitability in rearing this species. The current trend in fish feed is to formulate feeds with high energy and lipid content in order to obtain a "sparing effect" of proteins. In fact, protein being the most expensive component in fish feed, excessive protein content is wasteful and causes the diets to be unnecessarily expensive. High dietary energy levels must, however, be carefully evaluated as it may negatively affect carcass composition, mainly due to an increase of lipid deposition (Hillestrad and Johnsen, 1994).

The aim of the present work was to investigate the effect of a high energy content diet with two different protein levels on growth, protein utilization and body composition of sharpsnout sea bream.

Material and methods

Sharpsnout sea bream of 27.7 ± 0.2 g initial body weight were obtained from the hatchery Ittica Ugento, S.p.A (Lecce, Italy) and transported to the fish farm Novagriter scarl (Campomarino - CB, Italy). Fish were weighed, randomly divided in two groups of 300 fish per treatment and allocated into four outdoor 20 m^3 concrete circular tanks. Fish were acclimated to the experimental conditions for a week before starting the experiment. During this period, fish were fed a practical diet to satiation, (Europe Marine 1°, Skretting, Hendrix S.p.A., Mozzecane - VR, Italy) which presents a protein level of 48 % and a lipid level of 22%. The experiment lasted 94 days (July – October). Photoperiod and water temperature (26-20°C, average 24.0°C) followed natural cycles (14° 65' E 41° 57' N). Tanks were supplied with a continuous flow of natural seawater to maintain dissolved oxygen > 6 ppm while salinity (36 – 37 ppt) was monitored weekly.

Two experimental diets were formulated in order to obtain two different protein levels (46.7 and 52.5% dry weight) and an equal energy content (24 MJ Kg^{-1}), using commonly available ingredients (herring meal, fish oil, wheat meal, soybean meal, vitamin and mineral premix) and marked as LP and HP, respectively. The protein level was adjusted by replacing the protein ingredients with wheat meal. Diets were extruded to pellets of 2.8 – 3.2 mm die by Skretting, Hendrix S.p.A. Proximate composition is shown in Table 1. Feed was distributed automatically for three minutes per hour from 9.00 a.m. until 4.00 p.m. using a timer controlled disc feeder. During the automatic feeding, an identical amount of feed was portioned out to all tanks in order to reach an approximately daily ratio of 1.8% of the biomass. To avoid feed losses, an operator controlled fish appetite at the onset of feeding. The amount of automatically delivered feed was recalculated every week all through the experiment on the basis of a hypothetical growth and feed conversion rate at the size of the animals used in the trial. In the late afternoon (around 6.00 p.m.) feed was distributed a last time during

Table 1. Diet formulation and proximate composition.

		Diet LP	Diet HP
Ingredients (g $100g^{-1}$ wet weight):			
Herring meal		57.0	63.0
Fish oil		23.0	22.0
Wheat meal		14.0	9.0
Soybean meal		3.5	3.5
Vitamin and mineral premix		2.5	2.5
Proximate composition:			
Protein	g $100g^{-1}$ wet weight	46.7	52.5
Lipid	" "	31.1	29.2
Carbohydrates	" "	14.2	9.0
Ash	" "	7.8	9.0
Fiber	" "	0.8	1.1
Gross energy	MJ kg^{-1}	24.1	24.7
P/E ratio	g protein MJ^{-1}	18.3	20.8

the day by hand until satiety (i.e., until few pellets remain uneaten at the bottom of the tanks). The overall daily amount of feed distributed was recorded in order to measure daily intake rate (DIR). All dead fish were collected, weighed and recorded throughout the experiment. These weights were added to the final tank biomass for calculation of feed conversion rate (FCR). The effects of diets were determined by evaluating a number of growth and nutrient utilization indexes, including weight increment, specific growth rate, feed conversion rate (FCR), protein efficiency ratio (PER), gross protein efficiency (GPE) and gross lipid efficiency (GLE).

Before distributing the animals, 40 fish were killed by an overdose of phenoxyethanol, weighed and frozen at –20°C for subsequent analysis. At the end of the experimental period, after a 24h fast period, all fish were anaesthetized with phenoxyethanol (250 ppm) and weighed. Thirty fish per tank were randomly collected and used to measure individual weight and length, as well as liver and viscera weight for calculation of condition factor (CF), eviscerated yield (EY), hepatosomatic index (HIS) and viscerosomatic index (VSI). Fish proximate analysis was carried out on 20 animals per tank after homogenization in a mixer (PBI International). Dry matter of both animals and feeds was determined after drying samples for 24 h at 90°C. Crude protein (N x 6.25) was determined by the Kjeldahl method and total lipid using the method of Folch and Stanley (1957), starch by the glucose-amylase-glucoseoxidase method (Thivend *et al.*, 1972), crude fiber by digestion with 1.25 % H_2SO_4 and 1.25% NaOH solutions. Ash content was determined after combustion for 4 hrs at 550°C. Gross energy of feed was calculated using the following coefficients (Miglavs and Jobling, 1989): protein 23.6 kJ/g, lipids 38.9 kJ/g and carbohydrates 16.7 kJ/g. Student's t test was employed to compare data, using the software Graphpad Instat 3.05. In all statistical testing, differences at $P < 0.05$ were considered as significant.

Results and discussion

During the experimental period, mortality was low (0.5-1% $tank^{-1}$) and not related to the diets. Growth data are shown in Table 2. After a feeding period of 94 days, the weight gain of fish fed diet HP (122.1 ± 3.2 g) tended to be higher than that of fish fed diet LP (106.3 ± 6.5 g) with a specific growth rate (SGR) of 1.88 ± 0.03 and 1.75 ± 0.04,

Table 2. Effects of different protein levels on sharpsnout seabream growth.

		Diet LP	Diet HP
Initial weight	g	27.80 ± 0.3	27.60 ± 0.1
Final weight	"	134.10 ± 6.8	149.70 ± 3.1
Weight gain	"	106.30 ± 6.5	122.10 ± 3.2
SGR		1.75 ± 0.04	1.88 ± 0.03
DIR		1.59 ± 0.00[b]	1.65 ± 0.01[a]
FCR		1.12 ± 0.01	1.11 ± 0.01

a,b: $P < 0.05$
SGR (specific growth rate) = 100 x (ln final weight – ln initial weight)/days
DIR (daily intake rate) = (Dry food intake/[(Initial weight+final weight)/2]/ days) x 100
FCR (feed conversion rate) = g feed given / g live weight gain

Table 3. Effects of different protein levels on sharpsnout seabream somatic indices.

	Diet LP	Diet HP
CF	2.13 ± 0.17[b]	2.24 ± 0.25[a]
EY	90.40 ± 1.4	90.60 ± 2.1
VSI	9.27 ± 1.00	8.93 ± 1.00
HSI	2.46 ± 0.50	2.43 ± 0.51

a,b: $P < 0.05$
CF (condition factor) = (body weight/total length^3) x 100.
EY (eviscerated yield) = (eviscerated weight/body weight) x 100.
VSI (viscerosomatic index) = (viscera weight/body weight) x 100.
HSI (hepatosomatic index) = (liver weight/body weight) x 100.

respectively even though no statistical differences were noticed. FCR values were similar among groups (1.11 ± 0.01 and 1.12 ± 0.01). Daily intake rate (DIR) was higher for fish fed the HP diet than for those fed the LP diet ($P < 0.05$). Somatic indices are shown in Table 3. A significant difference ($P < 0.05$) was observed for condition factor (2.24 ± 0.25 and 2.13 ± 0.01 for diet HP and LP, respectively) whereas the other somatic indices were similar. At the end of the trial, the proximate composition of the whole body was similar among treatments (Table 4). Nevertheless, the final lipid percentage, high in both the groups (21.9 ± 2.2 and 22.2 ± 1.7 in diet LP and HP, respectively) was found significantly ($P < 0.05$) higher than that of fish at the beginning of the experiment. As a consequence, moisture content significantly decreased (P < 0.05) from 64.9 ± 2.1% at the beginning of the experiment to 57.9 ± 2.0 and 58.0 ± 1.1% at the end for diet LP and HP, respectively. The nutrient utilization indices are presented in Table 5. Protein efficiency ratio (PER) and gross protein efficiency (GPE) were statistically higher in diet LP (2.13 ± 0.01 and 33.4 ± 0.6) in comparison with diet HP (1.80 ± 0.01 and 28.0 ± 0.6). GLE values were similar among groups (75.5 ± 2.2 and 76.7 ± 0.5%).

Growth performances obtained in this trial can be considered comparable or better than those previously reported (Bermùdez *et al.*, 1989; Divanach *et al.*, 1993; Hernàndez *et al.*, 2001). Fish fed diet HP showed a slightly higher SGR than the other group (1.88 ± 0.03 *vs.* 1.75 ± 0.04) and a similar

Table 4. Proximate carcass composition (g $100g^{-1}$ wet weight).

	Initial	Final	
		Diet LP	Diet HP
Moisture	64.9 ± 2.1[a]	57.9 ± 2.0[b]	58.0 ± 1.1[b]
Protein	14.8 ± 1.7	15.5 ± 1.5	15.4 ± 1.2
Lipid	15.7 ± 2.1[b]	21.9 ± 2.2[a]	22.2 ± 1.7[a]
Ash	3.7 ± 0.5	3.6 ± 0.4	3.4 ± 0.2

a,b: P < 0.05

Table 5. Nutritive utilization of experimental diets.

		Diet LP	Diet HP
PER		2.13 ± 0.01[a]	1.80 ± 0.01[b]
GPE	%	33.40 ± 0.6[a]	28.00 ± 0.6[b]
GLE	"	75.20 ± 2.2	76.70 ± 0.5

a,b: P < 0.05
PER (protein efficiency ratio) = weight gain/protein intake.
GPE (gross protein efficiency) = (fish protein gain/protein intake) x 100.
GLE (gross lipid efficiency) = (fish lipid gain/lipid intake) x 100

FCR (1.11 ± 0.01 *vs*. 1.12 ± 0.01). This is due to the fact that fish fed diet HP consumed a higher amount of feed all through the experiment with a statistically higher ($P < 0.05$) DIR in fish fed diet HP than those fed diet LP. Many studies have been carried out to determine factors influencing food consumption in fish. In this trial, it would appear that fish may eat regardless of either energy or protein content of the diets. On the other hand, feed ingredients with low digestibility, such as carbohydrates, may potentially influence gastrointestinal emptying by increasing the volume of the digesta (Storebakken *et al.*, 1999) with a consequent decrease in feed intake. Krogdahl *et al.* (1999) observed a difference in an apparent starch absorption when salmon were fed a 16.5% carbohydrate diet in comparison with a 5.6% carbohydrate diet, the latter being better utilized showing double carbohydrate absorption. Grisdhale-Helland and Helland (1997) found an increase in feed intake in response to increasing dietary protein and decreasing carbohydrate levels in salmon fed every 5 min during the light period. The different composition of the experimental diets and, in particular, the carbohydrate content, may have affected gastrointestinal emptying, resulting in a higher appetite in fish fed diet HP at the last feeding made by hand, and a consequently higher consumption of food. This could also suggest that digestibility of ingredients, and thus gastric evacuation time, was able to influence feed intake at a greater extent when a high feeding frequency is applied.

Among somatic indices, condition factor revealed a statistically higher value in fish fed diet HP. This could be due to the fact that fish fed diet HP eating a higher amount of food, obtained a better development of flesh.

With respect to diet nutritional utilization, PER and GPE were statistically better ($P > 0.05$) in fish fed diet LP, indicating that the diet with 44.2% of protein and a P/E ratio of 18.3 g MJ^{-1} had been better utilized by juvenile sharpsnout sea bream under the present experimental conditions. In our study, the high lipid level in the diets could have determined a sparing effect of proteins, which was more evident in fish fed the lower protein level. According to Hernàndez *et al.* (2001), better growth rates in this species can be obtained by lowering the P/E ratio below 22 g protein MJ^{-1}, which could prompt fish to use fat and carbohydrate as non protein energy sources. The overall growth results obtained in this trial, suggest that this species is able to efficiently use high lipid levels (29-31%) and an even lower P/E ratio (18.3 and 20.8 g protein MJ^{-1}). According to Vergara *et al.*, (1999), the increase of dietary lipid levels up to 28% is needed to promote best growth in gilthead sea bream (*Sparus aurata*) when standard quality fish meal is utilized. On the other hand, the inclusion of 30% of dietary lipid did not modify growth rate but reduced protein and energy retention in *Dicentrarchus labrax* (Peres and Oliva-Teles, 1999), indicating that the common practice to administer an identical commercial feed both for sea bass and Sparidae should be carefully considered when a high lipid level diet is used.

The reduction of the dietary protein level for maximum growth by using a high level of dietary non-protein energy has been studied for different fish species, proving that in many cases the optimum level of dietary protein level can be reduced with the inclusion of higher lipid levels (Shian and Huang, 1990). In fact, albeit increases in dietary protein have often been associated with higher growth rates in many species (McGoogan and Gatlin III, 1999), it is known that there is a protein level beyond which, at a certain energy level, further growth is not supported so that excess protein is not used for anabolism processes but is catabolized to provide energy (Adron *et al.*, 1976, Lied *et al.*, 1982). Similar results have been reported for Sparus aurata, which may be considered closely related to sharpsnout sea bream. From an experiment on gilthead sea bream fed diets with graded level of crude protein (42-58%), Vergara *et al.* (1996) concluded that dietary protein level could be decreased from 58 to 46% when increasing the lipid content from 9 to 15%. They also observed a decrease in PER with the increase in protein in the diet. According to Santinha *et al.* (1999), when diets contained 15% lipid, increasing protein level from 47 to 51% led to an improvement in feed efficiency in sea bream (Sparus *aurata*) but there was no effect of protein level in 21% lipid diets.

Varying dietary protein level did not affect the percentage of body protein of sharpsnout sea bream. On the other hand, both dietary treatments significantly modified the body fat content of fish in comparison with fish at the beginning of the experiment, with a fat retention over 70%. In combination, moisture content significantly decreased from 64.9 to 57.9 (diet LP) and 58.0% (diet HP). The change in feed formula, in particular the higher lipid level used in the experiment clearly modified, as in other fish species (Bromley, 1980; Shimeno *et al.*, 1980; Weatherup *et al.*, 1997), the lipid content of fish but not protein and ash. Those data confirm what was found by Company *et al.* (1999b). They were able to demonstrate that protein and ash content were not significantly affected by protein levels of 55% and 46% in sea bream Sparus aurata fed to satiety while, increasing the dietary lipid level from 9% to 17%, a concomitant increase of body fat content was observed. Lanari *et al.* (1999), suggested that protein and ash in sea bass *Dicentrarchus labrax* seems more life–cycle and size dependent, while the fat of fish increases with energy intake and fish size.

Conclusions

Results of this trial indicated that, under the experimental conditions, juvenile sharpsnout sea bream consumed a higher amount of food when fed a 52.5% protein and 9.0% carbohydrate diet. This is probably due to a faster absorption of nutrients when carbohydrate level is lower. On the other hand, the 46.7% protein diet showed statistically higher, GPE and PER than 52.5% protein diet, giving a protein sparing effect, which is notable from a nutritional, environmental and economical point of view. The increase of whole body lipid content obtained at the end of the experiment must, however, be carefully evaluated when new feeds are formulated.

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