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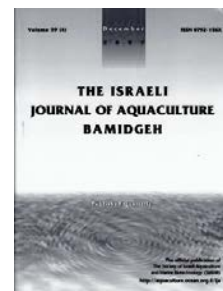
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## Effects of Dietary Lecithin, Nucleoside, and Krill Supplementation to a Fishmeal Based Diet on Growth and Feed Utilization of Sharpsnout Sea Bream (*Diplodus Puntazzo*)

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**Keywords:** *Diplodus puntazzo*; dietary lecithin; nucleosides; krill; specific growth rate; feed conversion ratio; daily feed intake; whole-body composition

### Abstract

The aim of this 96 day feeding trial was to investigate the effects of the addition of different combinations of dietary lecithin, nucleosides, and krill to a fishmeal-based diet on the growth, feed utilization, feed consumption, and body composition of sharpsnout sea bream (*Diplodus puntazzo*). Six hundred (600) fish (average weight  $21.21 \pm 0.06$ SD g) were divided into 8 groups (triplicate treatments) and fed eight isoenergetic fishmeal-based diets, (C-control, L-lecithin, N-nucleosides, K-krill, L+N-lecithin+nucleosides, N+K-nucleosides+krill, L+K-lecithin+krill, L+N+K-lecithin+nucleosides+krill). The effects of the dietary regimes were evaluated in terms of specific growth rate (SGR), feed conversion ratio (FCR), daily feed intake (DFI), and whole body chemical composition (moisture, crude ash, crude protein and crude lipid). At the end the trial the fish had tripled their initial weight. SGR, FCR and DFI were 1.17-1.24 %, 0.95-1.01, and 1.03-1.14% respectively. Although differences were observed between some groups, none of the tested feed additives improved SGR, FCR, and DFI, compared to the control diet. Analysis of whole body proximate composition showed that moisture, crude ash, crude protein, and crude lipid ranged 61.45-64.00%, 3.96-4.26%, 15.44-17.26% and 14.87-18.82% respectively. Crude lipid concentration was higher in whole body of

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fish fed the nucleoside supplemented diet compared to control, lecithin, and krill groups. No other effects of the dietary regime on the whole body composition of sharpsnout sea bream were observed.

## Introduction

Sharpsnout sea bream (*Diplodus puntazzo*) is a benthopelagic marine fish belonging to the Sparidae family exhibiting gregarious behaviour and omnivorous feeding. They are found from the Bay of Biscay to the coasts of Sierra Leone, the Mediterranean and Black Seas (Bauchot & Hureau 1986). Although sharpsnout sea bream have been cultured for over 30 years - albeit in small quantities in the Mediterranean Sea - knowledge of nutrient requirements derives from studies on protein and energy requirements (Hernández *et al.* 2001; Coutinho *et al.* 2012) and replacement of fish meal and oil by vegetable meal and oils (Hernández *et al.* 2007; Piedecausa *et al.* 2007) as well as studies dealing with feed preference and macronutrient selection by this species (Torrejón Atienza *et al.* 2004; Vivas *et al.* 2006; Almada-Pagán *et al.* 2008).

The present study was concerned with evaluation of the effects of feed additives, namely lecithin, nucleosides and krill on the growth and feed utilization of sharpsnout sea bream, as these additives had a positive effect on growth and feed consumption. Dietary lecithin has been effective in increasing growth of the larvae of fish such as pikeperch (*Sander lucioperca*) and juvenile rainbow trout (*Oncorhynchus mykiss*) (Tocher *et al.* 2008). It has also been suggested that lecithin could be a suitable substitute for fish oil in the diet of rainbow trout (Liu *et al.* 2004). Nucleosides have strong effects on diet palatability and feeding behavior and improve growth (Peng and Gatlin 2006). Krill, a feeding stimulant (Gaber 2005; Kader *et al.* 2010) has improved growth rates in fish such as Nile tilapia *Oreochromis niloticus*, (Gaber 2005), yellowtail *Seriola quinqueradiata*, (Kofuji *et al.* 2006), Atlantic salmon *Salmo salar*, (Olsen *et al.* 2006), Atlantic halibut *Hipploglossus hipploglossus*, and Atlantic cod *Gadus morhua*, (Tibbetts *et al.* 2011).

## Materials and Methods

600 fish (average weight 15-25g), obtained by means of mesocosm hatchery technology from a unique broodstock (Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, Crete, Greece), were selected for the experiment. The fish were collectively weighed and randomly distributed into 24 indoor 500L tanks (density of 25 fish per tank), with 3 replicates per treatment. Water was supplied from a borehole (salinity 39 ‰, temperature 21-22 °C). Light conditions in all tanks was identical (12:12). A net was placed over the tanks to prevent fish from escaping. Eight isoenergetic diets were formulated using different combinations of dietary lecithin, krill and nucleosides (Table 1).

**Table 1.** Composition (% dry matter basis) and chemical analysis (% dry matter basis) of experimental diets for *Diplodus puntazzo*. C: control; L: lecithin; N: nucleosides; K: krill.

Ingredient	Diet							
	C	L	N	K	L+N	N+K	L+K	L+N+K
Fish meal	68.0	68.0	68.0	61.0	68.0	61.0	61.0	61.0
Wheat flour	15.4	15.5	15.4	15.4	15.2	15.2	14.9	14.7
Wheat gluten	7.5	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Fish oil	7.0	6.0	7.0	5.0	5.8	5.0	4.0	4.0
Monophosphate	0.3	0.0	0.1	0.1	0.0	0.1	0.1	0.1
Premix Booster <sup>a</sup>	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Premix 2.5% <sup>b</sup>	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Stay-C	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Lysine	0.5	0.5	0.5	0.0	0.5	0.0	0.0	0.0
Methionine	0.3	0.0	0.3	0.0	0.3	0.0	0.0	0.0
Choline 60%	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Lecithin	0.0	1.5	0.0	0.0	1.5	0.0	1.5	1.5
Nucleosides	0.0	0.0	0.2	0.0	0.2	0.2	0.0	0.2
Krill	0.0	0.0	0.0	10.0	0.0	10.0	10.0	10.0
<i>Component</i>								
Crude protein	56	56	56	56	56	56	56	56
Crude lipids	13	13	13	13	13	13	13	13
Gross energy	20.5	20.5	20.5	20.5	20.5	20.5	20.5	20.5

a Vitamin A: 15000000 IU, Vitamin D3: 1000000 IU, Vitamin E: 300000 mg, Vitamin K3: 5000 mg, Vitamin B1: 38000 mg, Vitamin B2: 41000 mg, Vitamin B6: 29500 mg, Nicotinic Acid: 90000 mg, Pantothenic Acid: 40000 mg, Folic Acid: 6000 mg, Iron: 165000 mg, Copper: 4000 mg, Manganese: 80000 mg, Zinc: 175000 mg, Iodine: 1500 mg, Antioxidant: 25000 mg, Inositol: 350000 mg.

b Vitamin A: 10000000 IU, Vitamin D3: 2000000 IU, Vitamin E: 250000 mg, Vitamin K3: 10000 mg, Vitamin B1: 20000 mg, Vitamin B2: 20000 mg, Nicotinic Acid: 150000 mg, Pantothenic: 60000 mg, Vitamin B6: 20000 mg, Vitamin B12: 200 mg, Folic Acid: 4000 mg, Biotin: 800 mg, Vitamin C (Stay C) as Z-mannophosphate: 200000 mg, Inositol: 150000 mg, Manganese (Mn): 35000 mg, Iodine (I): 4000 mg, Iron (Fe): 45000 mg, Zinc (Zn): 60000 mg, Copper (Cu): 7000

All dietary ingredients for each experimental diet were thoroughly mixed in a feed mixer, moistened by adding 50% water, and then converted into pellets by a mincing machine. The pellets were manually cut and shaped, air dried at 35 °C for 24 hours, and stored in a freezer at -15 °C until used. The diets were randomly allocated to different tanks.

The entire experiment lasted 97 days and during this period the fish were hand fed twice a day (900am and 1500pm) until apparent satiation, taking care not to waste food. The fish were collectively weighed per tank at the beginning of the experiment, every month during the trial, and at the end of the experiment, after 24 h of starvation. They were slightly anaesthetized with 2-phenoxyethanol at a concentration of 150 ppm.

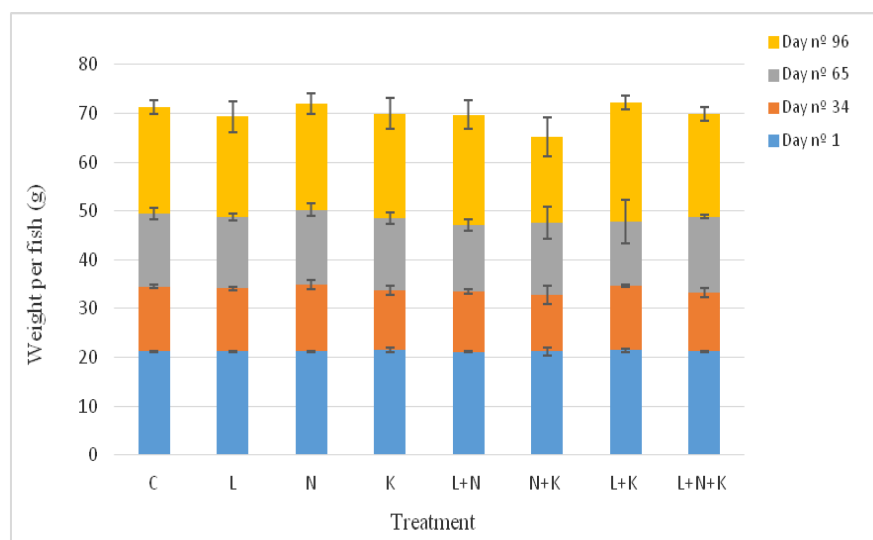
At the end of the experiment, the fish were euthanized with an overdose of anesthetic. A random subsample of 5 fish per tank was eviscerated to record hepatic mass. 5 more fish per tank were crushed for subsequent analysis of whole-body chemical composition. All samples were stored at -20 °C until the analysis. Following directives of AOAC (1997) proximate composition analysis, was performed: Moisture: dryness at 90°C until constant weight; Ash: burning in a furnace at 600 °C for 7 h; Crude proteins:

Dumas method with conversion factor 6.25 (Dumas JBA, 1831); Crude lipids: methanol/chloroform extraction (Folch *et al.* 1957). The indices of: specific growth rate ( $SGR = ((\ln(\text{final body weight} - \text{initial body weight})) / \text{days of the experiment}) \times 100$ ), feed conversion ratio ( $FCR = \text{feed consumed (g)} / \text{wet weight gain (g)}$ ), daily feed intake ( $DFI = (\text{daily feed consumed (g)} / \text{average total weight (g)}) \times 100$ ) and hepatosomatic index ( $HSI = (\text{liver weight (g)} / \text{whole body weight (g)}) \times 100$ ) were estimated for each treatment.

To find differences between treatments, non-parametric ANOVA and *post-hoc t-test*, using the statistical package SPSS v. 15.0 were conducted. The significance level selected was  $P < 0.05$ .

## Results

Over the feeding period the fish tripled their body weight reaching a final weight of c.a. 70g. Increase in the weight of fish over the whole experimental period is shown in Figure 1.



**Fig. 1.** Changes in weight per fish (sharpnose sea bream) with the different experimental treatments. L: lecithin; N: nucleosides; K: krill.

The indexes of SGR, FCR and DFI varied 1.17-1.24%, 0.95-1.01% and 1.03-1.14% respectively. No differences were observed in final fish weight, SGR, FCR and DFI between the control diet and the experimental diets. However, final fish weight was lower in the nucleosides+krill treatment compared to the nucleosides treatment and in the lecithin+krill treatment compared to the nucleosides+krill treatment (Table 2). SGR was lower with the krill treatment compared to the nucleosides treatment. FCR was lower with the nucleosides+krill treatment compared to the nucleosides treatment. No differences were observed in DFI and HSI among treatments.

Analysis of whole body proximate composition showed that moisture, crude ash, crude protein and crude lipid varied at 61.45-64.00%, 3.96-4.26%, 15.44-17.26% and 14.87-18.82% respectively (Table 3.) Lipid content of fish fed on the nucleoside treatment was higher compared to the control, krill, and lecithin treatments. The values of the remaining components, for proteins, moisture, and ash were not significantly different among diets.

Table 2. Weight (g), SGR (% per day), FCR, DFI (% per day) and HSI (% body weight) of sharpsnout sea beam fed on different additives. C: control; L: lecithin; N: nucleosides; K: krill.

Factor	Diet							
	C	L	N	K	L+N	N+K	L+K	L+N+K
Initial weight	21.21 ± 0.06	21.19 ± 0.10	21.23 ± 0.06	21.49 ± 0.51	21.16 ± 0.12	21.24 ± 0.85	21.47 ± 0.33	21.2 ± 0.05
Final weight	71.27 ± 1.41 <sup>ab</sup>	69.27 ± 3.22 <sup>ab</sup>	72.03 ± 2.07 <sup>ac</sup>	67.71 ± 1.11 <sup>ab</sup>	69.67 ± 2.92 <sup>ab</sup>	65.16 ± 4.07 <sup>b</sup>	72.19 ± 1.43 <sup>ac</sup>	69.77 ± 1.40 <sup>ab</sup>
SGR <sup>1</sup>	1.24 ± 0.03 <sup>ab</sup>	1.22 ± 0.05 <sup>ab</sup>	1.23 ± 0.01 <sup>a</sup>	1.17 ± 0.04 <sup>b</sup>	1.20 ± 0.06 <sup>ab</sup>	1.15 ± 0.03 <sup>ab</sup>	1.25 ± 0.02 <sup>ab</sup>	1.23 ± 0.02 <sup>ab</sup>
FCR <sup>2</sup>	0.95 ± 0.02 <sup>ab</sup>	0.93 ± 0.02 <sup>ab</sup>	0.95 ± 0.02 <sup>b</sup>	0.95 ± 0.02 <sup>ab</sup>	0.99 ± 0.07 <sup>ab</sup>	0.97 ± 0.05 <sup>a</sup>	0.98 ± 0.05 <sup>ab</sup>	1.01 ± 0.07 <sup>ab</sup>
DFI <sup>3</sup>	1.08 ± 0.02	1.07 ± 0.03	1.06 ± 0.03	1.03 ± 0.01	1.10 ± 0.05	1.05 ± 0.07	1.10 ± 0.05	1.14 ± 0.06
HSI <sup>4</sup>	1.86 ± 0.44	1.80 ± 0.31	1.88 ± 0.32	1.75 ± 0.27	1.98 ± 0.34	1.66 ± 0.48	1.45 ± 0.21	1.43 ± 0.27

<sup>1</sup> SGR: Specific Growth Rate; <sup>2</sup> FCR: Feed Conversion Ratio; <sup>3</sup> DFI: Daily Feed Intake; <sup>4</sup> HSI: Hepatosomatic Index

Values are means ± standard deviation. Different letters denote significantly different groups at  $p < 0.05$ .

Table 3. Whole-body composition of fish (%) from the different treatments after the experiment of sharpsnout sea beam. C: control; L: lecithin; N: nucleosides; K: krill.

Component	Diet							
	C	L	N	K	L+N	N+K	L+K	L+N+K
Moisture	64.00 ± 1.11	63.80 ± 2.27	62.09 ± 2.34	62.45 ± 1.31	62.98 ± 1.32	61.88 ± 1.42	61.31 ± 1.09	63.31 ± 1.60
Ash	3.96 ± 0.80	3.89 ± 0.53	4.00 ± 0.47	4.26 ± 0.78	4.14 ± 0.53	4.60 ± 0.93	4.12 ± 0.17	4.03 ± 0.15
Proteins	15.44 ± 0.69	16.30 ± 3.24	12.68 ± 3.17	17.55 ± 1.49	17.04 ± 1.03	18.33 ± 0.39	17.23 ± 8.76	17.26 ± 0.85
Lipids	14.99 ± 0.56 <sup>b</sup>	15.09 ± 0.37 <sup>b</sup>	18.82 ± 2.08 <sup>a</sup>	14.87 ± 0.42 <sup>b</sup>	14.53 ± 1.78 <sup>ab</sup>	15.80 ± 1.02 <sup>ab</sup>	15.98 ± 0.95 <sup>ab</sup>	15.21 ± 1.89 <sup>ab</sup>

Values are means ± standard deviation. Different letters denote significantly different groups at  $p < 0.05$ .

## Discussion

We found that dietary lecithin, nucleosides, and krill do not affect the growth and other biological parameters such as specific growth rate, feed conversion ratio, daily feed intake, hepatosomatic index and mortality of sharpsnout sea bream compared to a control fishmeal-based diet. Furthermore, lecithin and krill did not show any effect on whole-body content of moisture, ash, proteins and lipids.

A number of studies investigating the effect of dietary lecithin have shown an improvement in fish growth specifically with the larvae of pikeperch (*Sander lucioperca*). Levels of 9.5% of soybean lecithin in diet led to a 50% increase of larval final weight compared to a 1.4% inclusion level (Hamza *et al.* 2008). In juvenile of rainbow trout (*Oncorhynchus mykiss*), growth increased after a 14% soy-refined lecithin supplementation in diet (Rinchard *et al.*, 2007). In rainbow trout, when menhaden oil was replaced by 10 and 15% soybean lecithin, no differences in body weight were detected at the end of the trial (Liu *et al.*, 2004), suggesting that fish oil could be substituted by soybean oil. The beneficial effect of lecithin inclusion may depend on the developmental stage of fish as well as the composition of the diet under investigation. In the present feeding trial, sharpsnout sea bream were fed a complete fish meal diet. Growth and feed utilization did not improve after 1.5% lecithin supplementation.

Nucleosides have also been tested in diverse studies where their growth promoting effects have been seen in hybrid tilapia (Ramadan and Atef 1991) and rainbow trout (Adamek *et al.* 1996). It has also been shown to enhance immune response in hybrid tilapia, fed on a diet containing low fish meal (Shiau *et al.*, 2015).

\*\*On the other hand, no significant difference was found in growth performance of hybrid striped bass (*Morone chrysops* × *Morone saxatilis*) fed on a diet containing 0.5% of a commercial oligonucleotide (Li *et al.*, 2004). Nucleosides exert their growth promoting effect through enhancement of feed intake (Kubitza *et al.* 1997; Ikeda *et al.* 1991) and it appears that they did not further improve the already highly palatable fish meal diet in the present investigation.

Several studies on Nile tilapia (Gaber 2005), red sea bream (*Pagrus major*) (Kader *et al.* 2010) and black sea sash (*Centropristis striata*) (Alam *et al.*, 2012) have proved the beneficial effect of krill meal supplementation on fish diets since it acts as a feeding stimulant. Juvenile Nile tilapia (*Oreochromis niloticus*) fed with krill in increasing levels up to 6%, showed a significantly faster growth compared to fish meal based diets (Gaber 2005). Yellowtail (*Seriola quinqueradiata*) fed with diets containing 2% krill extract displayed faster growth performance than the control diet without feed stimulant inclusion (Kofuji *et al.* 2006). During the first period of feeding substitution of 20-60% of fish meal protein by krill protein, growth increased in Atlantic salmon (*Salmo salar*) compared to control fish (Olsen *et al.* 2006). After dietary substitution of fish meal by krill meal, Atlantic cod (*Gadus morhua*) and Atlantic halibut (*Hipploglossus hipploglossus*) also showed significantly higher growth rates (Tibbetts *et al.* 2011). In the present investigation inclusion level of 10% krill meal did not increase feed consumption and growth of sharpsnout sea bream. In fact, the krill and the nucleosides+krill treatments produced the lowest SGR values and the FCR of the nucleosides+krill treatment was higher compared to the nucleosides treatment. These observations might be related to the substantial amounts of chitin in krill meal. There is evidence that inclusion of chitin ( $\beta$ -1,4-linked N-acetylglucosamine residues) – the predominant polysaccharide in the organisms that form krill – in diets is negligible or restricts growth rates. Chitin lowered growth and feed utilization at 10% inclusion level in hybrid tilapia *Oreochromis niloticus* × *O. Aureus*, (Shiau & Yu 1999), while in freshwater fish cyprinids golden mahseer (*Tor putitora*) and snow trout (*Schizothorax richardsonii*) the inclusion of 2% chitin on the diet had no effect on growth rate (Mohan *et al.* 2009).

The differences in final body weight between the nucleosides treatment and the nucleosides+krill treatment as well as lecithin+krill and nucleosides+krill treatments did not correspond to differences in SGR and their biological significance is uncertain. Although the dietary treatments of krill and nucleosides caused differences in lipid concentration in the whole body proximate composition of sharp snout sea bream. The

biological significance of this observation is not clear (Table 3). The fact that body weight and whole body lipids of fish fed on nucleosides was higher compared to krill fed fish leads us to conclude that the difference in lipid content is related to the difference in body weight.

In conclusion, this feeding trial showed that dietary supplementation of krill meal, lecithin, and nucleosides, in a fish meal-based diet does not improve growth, feed consumption, and feed conversion ratio, in sharpsnout sea bream. Although the beneficial effects of these additives have been proven in fish meal or/and fish oil substitution studies, it appears that this may be masked by high palatability and high nutrient value of fish meal.

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