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THE EFFECT OF DIETARY SOYBEAN MEAL ON GROWTH, NUTRIENT UTILIZATION EFFICIENCY, AND DIGESTIBILITY OF JUVENILE COMMON DENTEX, *DENTEX DENTEX* (ACTINOPTERYGII: PERCIFORMES: SPARIDAE)

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Tomás A., Martínez-Llorens S., Jover M. 2009. The effect of dietary soybean meal on growth, nutrient utilization efficiency, and digestibility of juvenile common dentex, *Dentex dentex* (Actinopterygii: Perciformes: Sparidae). Acta Ichthyol. Piscat. 39 (1): 19–25.

Background. The aim of this work was to study the growth of juvenile dentex fed on cooking-extruded diets, determine the level of substitution of fishmeal by soybean meal may be without affecting the growth in this species and its digestibility.
Materials and Methods. The availability of defatted soybean meal as a substitute for fish meal was evaluated in juvenile (41 g on average) dentex by feeding diets containing 0%–60% soybean meal, for 97 days.
Results. Survival at the end of the experiment was high (80%) except for the fish fed diets with 50% and 60% substitution. Growth, feed gain ratio, and protein efficiency ratio (PER) were slightly reduced at higher soybean meal levels. Fish fed diets containing 0% to 40% of soybean meal grew significantly more and FCR was lower than fish fed other diets. No differences were obtained for protein digestibility coefficients of experimental diets (20%, 30%, 40%, and 50% SBM).
Conclusion. The results confirm the best protein level for optimum growth seems to be around 50% and 12% lipid level, and maximum soybean meal substitution of 40%.

Keywords: Dentex, protein sources, soybean meal, digestibility

INTRODUCTION

Intensive Mediterranean aquaculture consumes large amounts of food, between 1.5 and 2.5 kg per kg of fish produced. This food consumption represents around 45% of the variable costs of fish farms, which is why research into aquaculture feeding aims to reduce the feeding costs by optimising feeding strategies, either by reducing the price of diets through the optimisation of nutrient levels or by substituting fish meal with other less expensive protein sources. Likewise, and considering the limited availability of fishmeal, a sustainable increase in fish production must consider new protein sources.

Mediterranean aquaculture is developing an increasing interest in diversification of cultured fish species with the common dentex, *Dentex dentex* (L.), representing a possible candidate. It is a fast-growing sparid, highly appreciated by consumers and with an easy reproduction in captivity, although many problems remain with larval production (Abellán 2000).

Preliminary studies have shown that common dentex have high growth rates, (Riera et al. 1993, 1995, Efthimiou et al. 1994, Tibaldi et al. 1996, Abellán et al. 1997, Cardenete et al. 1997a, 1997b, Skalli et al. 1999, 2004, Espinós et al. 2003), but basic nutritional needs of juvenile dentex are practically unknown, because it is difficult to study this species owing to the important problems of stress and mortality.

Aquaculture research is currently seeking protein source substitutes for fishmeal, due to the increased cost and foreseeable market scarcity of this ingredient.

Researchers of Mediterranean species are focused on the use of plant meals (Robaina et al.1995, 1997, Watanabe et al. 1998, Aoki et al. 2000, Kissil et al. 2000, Pereira and Oliva-Teles 2002, 2003, Gómez-Requeni et al. 2004, Hernández et al. 2007, Martínez-Llorens et al. 2007, Sánchez-Lozano et al. 2007). However, plant meals yield lower protein levels and present other problems that limit their use in diets. For example, plants meals lack certain essential amino acids (methionine, lysine, and tryptophan) and contain antinutritional factors.

Soybean meals contain the highest amount of well balanced protein among the various oilseeds. Given its good amino acid profile, reliable supply and reasonable price, soybean meal is widely used in animal feeds. The most

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commonly used forms are the solvent-extracted, which have protein contents of either 44% or 48%, depending upon the presence of hulls. Although a number of anti-nutritional factors are present in soybean meal that may limit the ingredient's use, incorporation levels of 10%–40% have been reported for various sparids to reduce the cost of feeding and improve aquaculture sustainability has been researched by several authors. Inclusion of soybean meal for an other sparids like *Sparus aurata* (cf. Lupatsch et al. 1997, Kissil et al. 2000, Ceulemans et al. 2003), while for fish weighing more than 80 g a 50% dietary soybean meal can be used until the fish reach commercial weight, with no negative effects on growth or feed efficiency (Martínez-Llorens et al. 2007) and *Diplodus puntazzo* (cf. Rondán et al. 2004) tolerate 60% of soybean meal in their diet with no serious negative effects, aside from the need to increase feed consumption in order to attain similar growth values (Hernández et al. 2007).

Chatzifotis et al. 2008 studied the effect of partial substitution of fishmeal by soy protein concentrate (0%, 25%, and 40%) with taurine supplementation in juvenile dentex, and concluded that partial substitution of fish meal is possible with 25% of soybean concentrate without affecting growth, which can be improved further by dietary taurine supplementation of 2 g · kg^{-1} diet.

Negative effects on protein digestibility have been attributed to trypsin inhibitors and phytic acid of SBM. although they contain some antinutritional substances (Alarcón et al. 1999). Certain of those exert an influence on the process of digestion reducing nutrient digestibility. So, is important to study digestibility diets in SBM levels experiments.

MATERIAL AND METHODS

Culture system. The experiment was carried out in 14 cylindrical fibre tanks (1500 L) set up in a re-circulating marine water system (30-m^3 capacity) with a rotary mechanical filter and a gravity biofilter of ca. 36-m^3 capacity. All tanks were equipped with aeration and the water was heated by a heat pump installed in the system. Water temperature was 19.7ºC, salinity was 38.25 g · L^{-1}, dissolved oxygen was 6 ± 0.5 mg · L^{-1} and pH ranged from 7.8 to 8.5.

All tanks were equipped with aeration and the water was heated by a heat pump installed in the system. The photoperiod was natural in both trials and all tanks had similar light conditions.

Fish. Juvenile dentex from Greece were transported by truck and transferred into Aquaculture Laboratory of UPV (Universidad Politécnica) in Valencia. During acclimatisation period all fish were fed commercial diet with 45% crude protein and 20% of crude lipids (Aquaplus, Dibaq-Diproteg, S.A., Segovia, Spain).

After the adaptation period, 840 fish were redistributed into 14 cages (60 fish per tank), two for each diet, with 41 g of initial weight. The experimental period was of 97 days.

Specific growth rates (SGR) were calculated as

$$\text{SGR} (\% \ d^{-1}) = [(\ln W_f - \ln W\text{i}) / t] \times 100,$$

where:

t is the experimental duration [days].

Feed intake as FI (g 100 g · $fish^{-1}$ · day^{-1}) = 100 x feed consumption [g] / average biomass [g] × days. Feed conversion ratio (FCR) was calculated as $FCR = FI_{tot} / (W_f - W_i)$, where FI_{tot} [g] is the total feed intake per fish during the experimental period and protein efficiency ratio (PER) as PER = WG (weight gain) [g] / PI_{to} (g DM), where PI_{tot} [g] is the total protein intake per fish during the experimental period.

Diets and feeding. The composition diets and their proximate values are shown in Table 1 and Table 2 (Amino acid composition of ingredients and experimental diets). Fishmeal was replaced for soybean meal in 0%, 10%, 20%, 30%, 40%, 50%, and 60% SBM. Crude protein content was about 50% and lipid content was 18% in all experimental diets. Each experimental diet was assayed in duplicate.

Diets were prepared by cooking extrusion processing with a semi-industrial twin-screw extruder (CLEXTRAL BC-45). Processing conditions were as follows: 100 rpm speed screw, 110ºC temperatures, and 4053-5066-kPa pressure and 3-mm diameter pellets.

Fish were fed by hand twice a day (0900 and 1600 h) until apparent satiation. Pellets were distributed slowly, allowing all fish to eat. All fish were anaesthetized with clove oil at 30 mg · L^{-1}, containing 87% of euglenol (Guinama®, Valencia Spain) and individually weighed every 30 days.

Chemical analyses. Feed ingredients and diets were analysed by AOAC procedures (Anonymous 1990): dry matter (105ºC to constant weight), ash (combusted at 550ºC to constant weight), crude protein (N × 6.25) by the Kjeldahl method after an acid digestion (Kjeltec 2300 Auto Analyser, Tecator, Höganas, Sweden), crude lipid (hydrolysis with 6 N HCl prior to diethyl ether extraction in a Soxtec 1043 extraction unit, Tecator). All analyses were performed in triplicate.

Following the method described by Liu et al. (1995), total amino acids were analysed in a Waters HPLC system (Waters 474, Waters, Milford, MA, USA) composed of two 515 pumps, a 717 autosampler, a 474 fluorescence detector and a temperature control module. Approximately 50 mg of the experimental diets were respectively hydrolysed with 50 mL of 6N HCl with 0.5% phenol at 115ºC for 24 h. Aminobutyric acid was added as an internal standard before hydrolysation. The amino acids were derivatisated with AQC (6-aminoquinolyl-N-hydroxysuccinimidyl Carbamate). Methionine and cysteine were determined separately as methionine sulphone and cysteic acid after oxidation with perfomic acid. The amino acids were separated by HPLC with a C-18 reverse-phase column Waters Acc. Tag (150 mm × 3.9 mm).

Table 1

Ingredient content and proximate composition of experimental diets

Ingredient [g 100 g^{-1}]	0	10	20	30	40	50	60
Fish meal, herring (5-02-000)	67.21	60.9	54.57	48.28	41.95	35.62	29.32
Extracted soybean (5-04-604)	0.0	9.2	18.4	27.7	36.9	46.1	55.3
Wheat meal (4-05-268)	20.29	17.12	14	10.78	7.63	4.53	1.34
Fish oil (7-08-048)	11.5	11.76	12.01	12.28	12.55	12.78	13.03
Methionine	0.00	0.09	0.18	0.27	0.36	0.45	0.54
Vitamin-Mineral Mix[a]	1	1	1	1	1	1	1
Analysed composition [%]							
Crude protein	49.6	50.0	49.9	50.1	49.5	50.2	50.3
Ether extract	18.1	17.0	17.8	17.6	18.5	17.4	18.1
Crude fibre	2.7	2.9	3.3	3.5	3.8	4.1	4.4
Ash	9.9	11.3	10.3	10.7	10.8	10.3	9.9
Calculated values: N-free extract	19.7	18.7	18.7	18.1	17.3	18.0	17.1
Calculated values: GE (MJ kg^{-1})[b]	17.7	17.7	17.8	17.8	17.9	17.9	17.9
Calculated values: CP/GE (g kJ^{-1})	28.1	28.2	28.0	28.1	27.7	28.0	28.0

[a] Vitamin and mineral mix (values are g · kg^{-1} except those in parenthesis) Premix, 5; Choline, 2; DL-α-tocopherol, 2; ascorbic acid, 1; $(PO_4)_2Ca_3$, 1. Premix composition: retinol acetate, 1 000 000 IU · kg^{-1}; calciferol, 500 IU · k^{-1}; DL-α-tocopherol, 10; menadione sodium bisulphite, 0.8; thiamin hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides, 12; Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Try, 0.7 (Dibaq-Diproteg, S. A., Segovia, Spain).

[b] Calculated using: 23.9 kJ · g^{-1} protein, 39.8 kJ · g^{-1} lipid and 17.6 kJ · g^{-1} carbohydrate.

Table 2

Amino acids composition of ingredients and experimental diets

Raw material				Experimental diets						
	fishmeal	soybean meal	wheat meal	0	10	20	30	40	50	60
Amino acids										
Essential amino acids [g 100 g^{-1} dry matter]										
Histidine	2.41	1.75	0.25	1.38	1.37	1.34	1.32	1.28	1.27	1.25
Arginine	5.92	4.6	0.44	3.19	3.19	3.16	3.16	3.10	3.13	3.12
Threonine	3.38	2.4	0.31	1.87	1.85	1.81	1.78	1.73	1.73	1.71
Valine	3.62	3.07	0.45	2.02	2.04	2.03	2.03	2.01	2.03	2.04
Mehionine	1.93	0.63	0.12	1.16	1.09	1.00	0.92	0.84	0.78	0.71
Lysine	4.91	3.18	0.37	2.29	2.28	2.25	2.23	2.18	2.19	2.17
Isoleusine	3.19	3.18	0.37	1.85	1.89	1.90	1.94	1.94	1.99	2.01
Leucine	5.26	4.97	0.67	2.97	3.02	3.04	3.08	3.07	3.14	3.17
Phenylalanine	3.67	4.4	0.51	2.57	2.62	2.65	2.69	2.69	2.76	2.79
Non essential amino acids [g 100 g^{-1} dry matter]										
Cystine	1.24	1.32	0.38	0.67	0.69	0.71	0.73	0.74	0.77	0.79
Tyrosine	2.72	2.57	0.14	1.84	1.84	1.82	1.81	1.77	1.79	1.77
Aspartate	6.52	6.74	0.55	3.28	3.44	3.55	3.68	3.75	3.92	4.03
Serine	2.99	3.13	0.32	1.59	1.65	1.69	1.74	1.77	1.83	1.88
Glutamine	9.43	11.29	2.97	5.03	5.32	5.54	5.79	5.94	6.24	6.45
Glycine	5.26	2.82	0.46	2.78	2.70	2.58	2.49	2.37	2.31	2.23
Alanine	4.6	2.47	0.39	2.31	2.25	2.17	2.10	2.01	1.97	1.91
Proline	4.74	5.06	1.12	3.75	3.72	3.66	3.62	3.53	3.53	3.49
EAA/NEAA*	0.91	0.80	0.55	0.91	0.90	0.88	0.87	0.86	0.85	0.84

*EAA/NEAA, essential amino acids/non-essential amino acids.

Digestibility. Apparent dry matter and protein digestibility coefficients of experimental diets were determined using four cylindroconical faeces collection tanks (250 L) with one settling column. Trial was carried out according to a Latin square design, so all fish were fed all diets (four replicates per diet); each feeding period was of around 22 days in order to collect an appropriate quantity of faeces.

Water temperature was 21 ± 0.5ºC. Water quality parameters including dissolved oxygen, pH, NO_2, and NH_4^+ were monitored regularly and were in the following ranges: O_2, 6–7 ppm; pH, 7.6; $NO_2^- < 0.5$ mg · L^{-1} and $NH_4^+ < 0.1$ mg · L^{-1}.

Apparent digestibility coefficient of nutrients was determined by the indicator method using acid insoluble ash (AIA) as an inert marker.

Four of the seven diets of growth experiment were tested in the digestibility trial, 20%, 30%, 40%, and 50% SBM, because the worst results of diet 60% and the low level of soybean meal of 0% and 10% with similar growth with others tested diets.

Fish weighing 105 g of initial mean weight were fed once a day (1200 h) at 1.5% feeding rate, and accumulated faeces were withdrawn before feeding, centrifuged and kept at –20ºC until analysis. Dry matter and protein of faeces were analysed using the same method as diets, which has been briefly described in an earlier paragraph (Anonymous 1990) and for AIA content by dissolving the remaining ash after incineration in hydrochloric acid (Anonymous 1981).

Apparent digestibility coefficients of nitrogen in the diets were calculated as

$$ADC_X = (1 - AIA_{diet} / AIA_{faeces} \times X_{faeces} / X_{diet}) \times 100$$

where: X represents nitrogen, AIA_{diet} and AIA_{faeces} are the AIA content (% dry matter) in the diet and faeces, respectively and X_{diet} and X_{faeces} are the quantity of X in 1 g dry matter of the diet and faeces, respectively.

Statistical analysis. Growth data and nutritive parameters were treated using multifactor analysis of variance (ANOVA), introducing the initial live weight as covariate (Snedecor and Cochran 1971). Newman-Keuls test was used to assess specific differences among diets at 0.05 significant levels (Stat graphics, Statistical Graphics System, Version Plus 5.1, Herndon, Virginia, USA).

RESULTS

The results of the present study clearly show that the growth and nutritive value of dentex may be differently affected when differently soybean levels are used to replace fishmeal protein. Clearly, the dietary amino acids varied considerably among diets (Table 2). Dietary histidine, threonine, methionine, and lysine increased in accordance with the dietary level of soybean meal. However, isoleucine, leucine, and phenylalanine content in diets were lower as the soybean meal content increased.

During the first month of the test the survival of the fish fed with the different diets was normal, but in the following months a great mortality took place, mainly of the fish that were consuming the 50% and 60% diets. At the end of the test the survival rates were quite variable, 80% for diets 0%, 10%, 20%, 30%, and 40%, 38% for diet 50% and 29% for diet 60% substitution (Table 3).

Table 3

Effect of soybean meal substitution on growth and nutritive parameters of dentex

Diets	0	10	20	30	40	50	60
Initial weight [g]	41.7 ± 14.5	40.9 ± 14.5	42.7 ± 14.5	42.2 ± 14.5	40.2 ± 14.5	42.8 ± 14.5	39.5 ± 14.5
Final weight [g]	97.4^{a} ± 3.9	98.3^{a} ± 3.9	90.9^{a} ± 3.9	89.5^{a} ± 3.9	78.8^{ab} ± 4.0	65.7^{b} ± 5.9	62.2^{b} ± 4.0
SGR [% day^{-1}] [t]	0.90^{a} ± 0.04	0.92^{a} ± 0.04	0.83^{a} ± 0.04	0.81^{a} ± 0.04	0.67^{ab} ± 0.04	0.57^{bc} ± 0.06	0.43^{c} ± 0.04
Survival [%]	78.0^{a} ± 9.3	81.5^{a} ± 9.3	82.0^{a} ± 9.3	79.5^{a} ± 9.3	80.0^{a} ± 9.3	38.0^{b} ± 16.2	29.5^{b} ± 16.2
FI [u]	0.97^{abc} ± 0.04	1.05^{a} ± 0.04	0.97^{abc} ± 0.04	0.93^{abc} ± 0.04	1.02^{ab} ± 0.04	0.80^{abc} ± 0.05	0.77^{c} ± 0.04
FCR [v]	1.32^{a} ± 0.25	1.42^{a} ± 0.25	1.38^{a} ± 0.25	1.69^{a} ± 0.25	2.34^{b} ± 0.25	2.08^{ab} ± 0.25	3.17^{b} ± 0.25
PER [x]	1.53^{a} ± 0.08	1.41^{a} ± 0.08	1.46^{a} ± 0.08	1.18^{ab} ± 0.08	0.86^{bc} ± 0.08	1.02^{b} ± 0.11	0.64^{c} ± 0.08

Values are means ± standard deviation from duplicate groups of fish, and the means in each row with the same superscript are not significantly different (Newman-Keuls);

[t]Specific growth rate [% / day]. SGR = ln (Final weight / Initial weight)/days.

[u]Feed intake [% lw] FI = 100 × Feed consumption [g] / average biomass [g] × days.

[v]Feed conversion ratio. FCR = Feed consumption [g] / Biomass gain [g].

[x]Protein efficiency ratio. PER = Biomass gain [g] / Protein intake [g].

The evolution of weight of the fish fed with the seven diets in this trial is shown in Fig. 1. Differences can be observed from the second month of the experiment; dentex fed the diets with substitution levels starting from 40% (40%, 50%, and 60%) had lower weights than those of the other diets.

In this experiment, feeds were offered to visual satiety and relative to control, palatability was adversely affected by level of soybean meal inclusion in the diet, the feed intake was inferior in dentex fed diets 50% and 60% SBM.

All growth and nutritional parameters were influenced by the level of substitution of fishmeal by soybean meal (Table 4). The final weight, SGR and FCR were significantly better in the fish fed the diets with a rate of substitution of fish meal by soybean meal from 0 to 40%. The PER was significantly higher in the diets with a substitution rate of 0% to 30% and the FI of 0% to 40%.

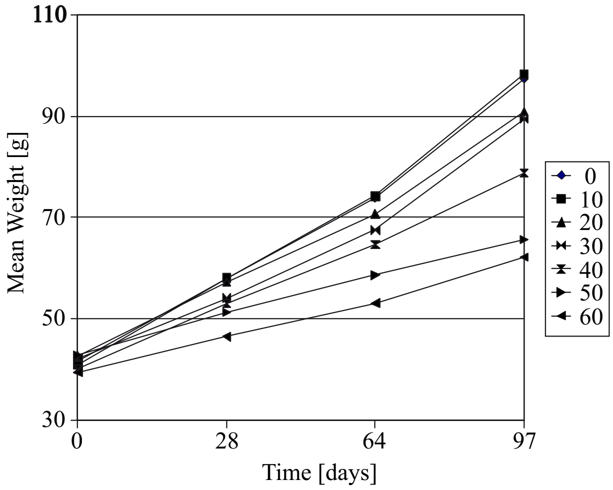

Fig. 1. Evolution of mean weight of dentex fed different dietary soybean meal levels

Table 4

Apparent digestibility coefficients (ADCs) of experimental diet components

ADCs [%]	20%	30%	40%	50%
Dry matter	65.6 ± 4.6	56.6 ± 4.6	61.7 ± 4.6	53.1 ± 4.6
Protein	91.9 ± 2.0	89.9 ± 2.0	89.9 ± 2.0	93.1 ± 2.0

Values are means ± standard deviation from quadruplicate groups of fish, and the means in each row with the same superscript are not significantly different (Newman-Keuls).

No effect of SBM inclusion level was on ADCs of diets (Table 4). This apparently resulted from the fact that digestibility measured for the first level of soybean meal were similar or better than those of the control diet.

DISCUSSION

During the experimental period of this experiment, a high mortality was observed in the dentex fed diets containing 50% and 60% substitution, probably due high levels of soybean meal affecting the palatability, since feed intake was lower when soybean meal content was up to 40%.

In this experiment, significant differences were observed for the final weight, specific growth rate, feed conversion ratio and protein efficiency ratio.

The best growth results were obtained as of 40% substitution; starting from this substitution level the final weight values, TCI, IC, and CEC worsen progressively, which may be due to a smaller percentage of fish meal in the diet or to the fact that the fish fed with the 50% and 60% had a smaller feeding rate. The growth reduction with soybean meal could be caused by amino acid profile imbalance of diets and the lower methionine and lysine content in diets with soybean.

Therefore, common dentex can tolerate a fishmeal substitution by soybean protein of 40% without affecting growth and feed utilization. This level is higher than in sea bream (Martínez-Llorens et al. 2007) with growth worse up to 30% soybean meal with fish weighing less than 80 g, and lower than sharp-snout sea bream, which tolerate 60% of soybean meal in the diet with no serious negative effects on growth, probably due an omnivorous nutrition.

These results are contrary to Chatzifotis et al. (2008) who obtained low growth and worse feed utilization with 40% substitution level from 20% for 40 g fish.

Palatability problems were apparent for any of the diets in the present study. Tomás et al. (2005) and Venou et al. (2006) reported increased consumption with increasing SBM inclusion in their study (0%–50% SBM). The increase in feed consumption could result from reduced available energy content as SBM inclusion increased, but calculation of the digestible energy content of the diets indicated similar with SBM inclusion (Table 1). The contrary it happens in present work, as SBM level increase the FI decrease, it seems dentex has worst palatability with diets increasing SBM diets.

Digestibility of dietary protein in test diets fed to dentex ranged from 89% to 93%, with no significant effect of soybean meal level. These findings are in agreement with digestibility studies for a range of species: silver perch (Allan and Booth 2004), yellowtail (Tomás et al. 2005), juvenile haddock (Kim et al. 2007), sharpsnout seabream (Hernández et al. 2007), European sea bass (Tibaldi et al. 2006) and gilt-head sea bream (Venou et al. 2006).

Protein digestibility was not affected by level of SBM in accordance with a number of studies (Tomás et al. 2005, Venou et al. 2006, Kim et al. 2007) for different fish species. This could be attributed to the generally low values of trypsin inhibitors and phytic acid resulting from the heat treatment applied during thermal processing of commercial SBM and extruded diets. However other authors have observed a decrease in protein digestibility (Tibaldi et al. 2006 and Hernández et al. 2007) and attributed mainly to the presence of phytate.

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