INCORPORATION OF LUPIN SEED MEAL AS PLANT PROTEIN SOURCE IN GILTHEAD SEA BREAM (Sparus aurata) DIETS.

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### ABSTRACT

The extruded lupin seed meal (Lupinus albus) was used as alternative protein source to partially replace fish meal in feeding of Sparus aurata. Four experimental diets were formulated to contain 40.68% ±0.12 CP. Diet 1, without lupin seed meal and considered to be the control, where L10, L20 and L30, lupin seed meal was incorporated at 10,20 and 30%, respectively. Sixty fingerlings of Sparus aurata with an initial body weight of 5.45±0.05g were distributed at random in twelve fiber glass tanks (each of 1 m<sup>3</sup>). The fish were fed 3% of their total body weight and the diets were offered at two times/day. Growth performance of fish showed a significance differences (P<0.05) between treatments. The highest performance was recorded with the fish fed 20% lupin seed meal (L20), followed with lesser extant by the control and L10 diets, respectively. However, the least performance was recorded with 30% incorporation level of lupin seed meal (L30). The carcass composition of fish were not affected by the incorporation level of lupin. The digestibility coefficients of the experimental diets showed a good utilization for protein in all tested diets. However, the carbohydrate digestibility coefficients recorded inferior results. On the other hand, the blood characteristics of fish (Hematocrite, Hemoglobin and plasma protein content) not differently by lupin seed level in the diets. The results of the present trial concluded that, the lupin seed meal can be utilized at level of 20% in feeding of Sparus aurata, without adversely effects on growth performance, digestibility coefficient and blood characteristics.

Keywords: Fish- Growth performance- Carcass composition- Amino acid- Fatty acid-Digestibility coefficient- Lupinus albus - Sparus aurata.

# INTRODUCTION

Replacement of fish meal with protein rich plant ingredients in fish diets has been the objectives of numerous recent nutritional studies. However, fish meal and plant protein sources differ in a number of ways including protein quantity, amino acid profile, energy density and mineral content. Plant proteins also contain one or more of anti- nutritional factors (ANFs), which may have adverse effects on both nutritional value and palatability (Kaushik, 1989)

The supply of different protein sources (meat meal, fish meal and particularly soybean was revised. Among these, cultivated lupin- species (*Lupinus L.*) have good potential due to their high protein concentration (30-40%), promising oil content (6-12%), and their similarity in composition to that of soybean. So lupins could substitute for soybean. Moreover, it can be grown in different soils, different environmental conditions and under conditions not suitable for soybean (Gladstones, 1970).

Lupins as grain legumes, are attracting attention throughaut the world as a potential providers of high quality protein and fat for future (Lopez Bellido and Fuentes, 1986). A factor which could limit the acceptance of lupin seeds for consumption is undoubtedly the presence of toxic alkaloids. The alkaloids concentration may, however, be reduced by subjecting the seed to heat treatment and rinsing with water. The oil content of lupin is too low to warrant extraction, but it makes a valuable contribution to the metabolisable energy value of the seed (Hansen and Zochanska, 1974). Lupin seeds appear to be free of the major anti-nutritional factors, trypsin inhibitors and haemagglutanins (Hill, 1977 and Hudson, 1979) and are fed without heat processing. Newer, "sweet" variets are virtually free of alkaloids (< 0. 1g/kg, Batterham et al., 1986).

Several researches investigated the use of lupin seed meal in fish feeding. Robaina et al., (1995) have shown that lupin meal was a suitable ingredient in diets for gilthead sea bream. Workers with rainbow trout De la Higuera et al., 1988 and Hughes, 1988 & 1993) have reported that incorporation of between (30 and 40%)dietary lupin meal did not affect on growth performance. Yet De la Higuera et al. (1988) noted a decrease in feed intake at 30% lupin incorporation. In fact, the palatability of lupin may be affected because it contains quinalizadine alkaloids (Hill and Pastuszewska, 1993). However, over the past decade, plant breeders have developed some strains of white lupin (*Lupinus albus*) with lower content of alkalaids (Roemer, 1983). Also, an advantage of lupin is that not require heat treatment as shown by De la Higuera et al. (1988) and Hassanen (1998), because it does not contain haemagglutanins (lectins) or trypsin inhibitors.

The present study was carried out to determine the nutritional value of extruded lupin (*L. albus*) in feeding of gilthead sea bream (*Sparas aurata*), besides, growth performance nutrient utilization, apparent digestibility coefficient and blood characteristics of sea bream (*Sparus aurata*).

## MATERIALS AND METHODS

#### Fish and experimental condition

This study was conducted using the research facilities of the experimental station at Shakshouk, Fayaum Governorate, south west of Egypt. Gilthead sea bream (*Sparas aurata*) fingerlings were obtained from the Mediterranean sea coast at Damietta in the northern east of Egypt. They were transported in convenient tanks with aeration system to the experimental station.

The experimental fish were randomly distributed and stocked at density of 60 fish with an initial average weight of 5.45±0.05g per fiber glass tanks (1m³), containing filtered sea water and provided with aeration. The water was delivered at a rate of 300 L/tank/hr.

The system contained two water pumps and upstream sandy filter units at a point between the water source and tanks. Each pump drew the water from Qarun lake and forced it through the body of the tanks and storage units. The fish were fed the experimental diets for two weeks as an

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acclimatization period. The experimental period lasted 120 days (from May to August 2002). Physicochemical characteristics of water tanks were examined every week according to APHA (1992).

### Diets and digestibility measurements.

Lupin albus L. Was prepared to be used as a plant ingredient for diets of Sparus aurata. They provided by Agriculture Research Center. After crushing, seeds were separated from its hulls. The rough flours obtained was extruded by extruding system had the following characteristics: temperature 127°c, pressure of 38 bar and specific energy of 89.8 w/kg. The chemical composition of lupin for protein and amino acids content is presented in Table (1). While, the fatty acid profiles were shown in table (2).

Table (1): Chemical composition and essential amino acids of lupin seed meal (*Lupinus albus*) and soybean meal (*Glycine max*)

Chemical composition:	Lupin seed meal	Soybean meal
Dry matter	92.8	91.4
Crude protein	40.0	49.7
Crude fat	10.0	1.4
Nitrogen free extract	32.6	34.5
Crude Fiber	12.8	5.1
Ash	4.6	9.3
Total alkaloid	0.22	-
Essential amino acids:		
Arginine	5.12	3.67
Histidine	1.34	1.22
Isoleucine	1.64	2.14
Leucine	3.12	3.63
Lysine	1.82	3.08
Methionine	0.34	0.68
Cystine	0.69	0.75
Phenylalanine	1.51	2.44
Tyrosine	2.12	1.78
Tryptophan	0.43	0.69
Valine	1.82	2.55

Table (2). Fatty acids composition of % of total seed mixture.

Saturated fatty acids:	Lupin seed meal	Soybean meal	
Palmitic acid (C16: o)	7.18	0.98	
Stearic acid (C18: o)	2.11	3.88	
Arachidonic acid (C20:o)	0.7	0.25	
Behenic acid (C 22:o)	4.38	1.9	
Unsaturated fatty acids:			
Oleic acid (C18:1)	48.78	36.2	
Linolic acid (C18:2)	19.91	36.3	
Linolenic acid (C18:3)	12.64	49.7	
Erucic acid (C 22: 1)	2.23	0.2	

Four isonitrogenous diets were formulated to containing average crude protein of  $40.68\% \pm 0.12$ . Each diet had three replacets. The diets were given at 3% of body weight (BW) per day at two times day (at 10.00 and 16.00 h) in two equal portions. The diets formulation is presented in Table (3). The first diet without extruded lupin was considered as a control diet (C). However diets 2 (L,10),3 (L20) and 4 (L30) containing extruded lupin with an incorporation levels of 10, 20 and 30%, respectively. All the diets were made into dry sinking pellet, using California pelleting machine with 3mm diameter.

During the last week of the experiment, fishes were fed on the experimental diets after adding 0.5% chromic oxide to study the apparent digestibility coefficients (ADC) of nutrients. During the collection period (10 days) for each diet, fecal samples were collected using the filtration system developed by Choubert et al., (1982)and frozen daily. After freeze- drying the Feces were analyzed. The ADC values of nutrients were calculated using the formulation of Maynard and Loosli. (1969).

Chemical analysis of ingredients, feces and carcass composition.

Analysis of ingredients, diets, freeze dried feces and carcass samples were analyzed according to AOAC (1995). Chromic oxide in diets and feces was determined according to Bolin et al., (1952). For amino acids analysis, the ingredients and diets were hydrolyzed with 6 N HCl at 110 c° for 24h for the chromatographic separation and analysis of the amino acids using a high performance liquid chromatograph (HPLC) as described by Gardner and Miller (1980). Tryptophan was determined calorimetrically in alkaline hydrolysate according the method described by Blauth et al. (1963).

For fatty acids, total lipids were extracted according to Folech *et al.* (1957). Fatty acid methyl esters were prepared by acid — catalyzed transmethylation of total lipids (Shantha and Ackman, 1990). They were analyzed in a varian 3400 gas chromatograph (equipped with a DB. Wax fused capillery colum (30 mx 0.25 mm i.d, film thickness, 0.25 Mm , Jw, USA) using helium as carrier gas (1.2 ml / min) and thermal gradient from 180to 240 °c/ min. injector and flam ionization detector temperature were 260 and 250 °c, respectively. Data were recorded on a spectra physics 4270 integrator. Identification of individual fatty acid was made by comparison with known standard mixture).

Total alkaloids of lupins were determined gravimetrically according to the methods described by Priddis (1983), the dominant alkaloids in extracts obtained from defated mixture of the particular varieties were identified by thin layer chromatography. Alkaloid extracts were separated on silica gel plates (Merck) using chloroform, cyclohexane diethylamine (6: 4: 1) as the solvent system. The alkaloid standards used were Lupanine perchlorate isolupanine and 13- hydroxylupanine (Koch- Light ltd Colnbrook, UK). The concentrated alkaloids were dissolved in 1ml of methanol Mierek silica gel plates (60F- 25420 ×20) and cyclohexane: diethylamine (1:10 were used.

The gross energy content of the diets was calculated as 5.5, 9.19 and 4.1 Kcal/ g of protein, lipid and carbohydrate, respectively according to Jobling (1981).

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The metabolizable energy contents of the experimental diets were calculated as 4.5, 8.5 and 3.49 Kcal/g of protein, lipid and carbohydrate, respectively according to Jauncy (1982b).

#### **Blood** analysis

Blood samples were drawn from the caudal vein with heparinized syringes. Hematocrite percentage and hemoglobin concentration were determined according to Harding and Hoglund (1983). Plasma protein, glucose, triglyceride and phospolipid were determined according to Shimeno et al. (1981)

### Statistical analysis

The analysis of variance (ANOVA) was employed to test the effect of lupin incorporation on various growth parameters, digestibility coefficients and blood characteristics according to Snedecor and Cochran (1987). Duncan multiple range test was used to detect the significant differences between the means of treatments (Duncan, 1955).

#### RESULTS AND DISSCUSSION

Results of the chemical composition of lupin seed meal (*L. albus*) compared with soybean meal (*Glycine max*) are shown in Table (1). The Lupin showed a good protein content (40.0%) and high content of fiber (12.8%) compared with soybean meal. This strain of lupin also showed a lower content of total alkaloid (0.22%). Similar values of amino acids were found in the two crops, with few exception.

As presented in Table (2), the fatty acid contents in *Lupinus albus* are comparable to that of soybean, except that the Oleic acid and Erucic acid were higher in *Lupins albus*. However, the linolic and linolenic acids showed higher values in soybean compared with *L. albus*.

Data presented in Table (3) showed that the crude protein in the experimental diets ranged from 40.5 to 40.8% and these values within the recommended values (40%) of this fish species (Sabout and Luquet, 1973).

The amino acid contents of the experimental diets were presented in Table (4). All diets having the essential amino acids required for rearing *Sparus aurata* as recorded by Jauncy (1988).

In Table (5), the physicochemical characteristics of tanks water showed good values for temperature, PH, dissolved oxygen, salinity and unozied ammonia. The recorded values are within the optimum ranges for rearing *Sparus aurata*. According to Porter *et al.* (1986).

As given in Table (6), the growth performance of fish fed the experimental diets showed significant differences (P< 0.05) among diets. The highest final body weight and gain were obtained with fish received diet L20, which contain 20% extruded lupin seed meal as an incorporation level followed (at a decreasing order) by the control diet and that contain 10% extruded lupin seed meal. However, the incorporation of 30% lupin meal showed less final body weight and gain in weight compared with the other

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diets. The highest growth rate (GR) and specific growth rate (SGR0, were recorded with diet L20, followed by the control and L10, respectively without significance difference among them. The (GR) rate and (SGR) were obtained with 30% incorporation of lupin (L30).

Table (3). Formulation and chemical composition of the experimental diets.

Ingredients %	Control	L10%	L20%	L30%
Fish meal	40	30	20	15
Lupin seed meal	-	10	20	30
Poultry -by-product meal	15	20	25	25
Gluten meal	10	10	10	10
Maize	20	15	10	5
Wheat bran	10	10	10	10
Fish oil	3	3	3	3
Vitamin/ Mineral Mix <sup>1</sup>	2	2	2	2
Chemical composition (%DM b	asis)			
Dry matter	92.5	91.4	29.0	92.2
Crude protein	40.8	40.65	40.5	40.8
Ether extract	15.39	15.2	16.24	15.4
Nitrogen free ext.	25.71	26.79	25.89	25.2
Fiber	4.78	5.07	6.09	8.51
Ash	13.32	12.29	11.28	10.09
Calcium	1.5	1.40	1.35	1.30
Phosphorus	0.8	0.86	0.75	0.70
Gross energy kcal/kg diets	4098.1	4717.2	4766.7	4678.6
ME Kcel/Kg diet	4041.3	4053.3	4106.4	4024.4
P/E ratio, mg Kcal	115.14	116.04	117.69	114.67

<sup>1-</sup> Vitamin mineral premix, each Kg contain: vitamin A, 2500 IU, Vitamin C, 6.0g (ascorbate polyphosphate), Vitamin D3, 2400 IU,∞ tocopheryl acetate 0.2 g, thiamin, 0.01g (thiamin cl), riboflavin 0.02g, pyridoxine 0.01g, (pyridoxine Cl), Ca- pantothenate 0,04g, niacin 15g, folic acid, 5mg vitamin k, 0.04g, Mn,35mg, (MnSO4), Zn, 90mg (Zn2SO4), cu, 12mg (CuSO4), I, 2mg (KI), Se,0.2mg (Na2seO3), Cl,1.25g KCl). The ingredients and vitamin mineral premix were obtained from Zoocontrol company, 6 October Giza – Egypt.

Table (4). Essential amino acid composition of the experimental diets (g/100g diet).

Amino acid		Diets			
	Requirement*	Control(C)	L10	L20	L 30
Arginine	2.11	3.03	3.14	3.31	3.54
Histidine	0.89	1.2	0.91	0.97	1.03
Isoleucine	1.37	1.4	1.5	1.59	1.65
Leueine	2.5	3.16	3.30	3.44	3.53
Lysine	2.90	3.2	3.12	3.05	2.98
Methionine+ cyst	1.28	1.39	1.32	3.24	3.25
Phenylanine + Tyrosine	2.25	3.37	3.25	3.24	3.25
Threonine	1.58	1.73	1.62	1.65	1.46
Tryptophane	0.29	0.54	0.52	0.49	0.46
Valine	1.63	1.84	1.93	1.94	1.73

<sup>\*</sup> According to Jauncy (1988).

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Table (5). Average values of the physicochemical characteristics of water in the experimental tanks throwout the experimental period.

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D		Diets					
Parameters		Control	L,10	L20	L30		
Temperature	°C	24.5	24.8	24.7	24.6		
PH		7.6 ±0.2	7.5± 0.3	7.6±0.1	7.5±0.2		
Dissolved ox	ygen (mg/l)	6.5±0.1	6.4±0.2	6.5±0.1	6.3±0.1		
Salinity ‰		35.1 ±0.1	35.2 ±0.2	35.1 ±0.1	35.1 ±0.1		
Unionized (mg/L)	ammonia	0.034± 0.002	0.032±0.001	0.032±0.002	0.034±0.001		

Table (6): Growth performance of Sparus aurata fed the experimental diets.

Deremeters		Diet	S		
Parameters	Control	L,10	L20	L30	SE±
Initial aveg. weight (g/fish)	5.5ª	5.4ª	5.4ª	5.5ª	0.05
Final aveg. weight (g/fish)	57.4 <sup>ab</sup>	55.8 <sup>b</sup>	60.2ª	45.8°	2.33
Gain (g/fish)	51.9 <sup>ab</sup>	50.4 <sup>b</sup>	54.8ª	40.3°	0.06
Feed consumed (g/ fish)	92.0	90.0	85.0	80.0	-
Growth rate (g/day)	043 <sup>a</sup>	042 <sup>a</sup>	045 <sup>a</sup>	033 <sup>b</sup>	0.04
Specific growth rate	1.95 <sup>a</sup>	1.95ª	2.0ª	1.76 <sup>b</sup>	0.09
Feed conversion <sup>2</sup>	1.77 a	1.78 <sup>a</sup>	1.55ª	1.98 <sup>b</sup>	0.15
Feed efficiency <sup>3</sup>	0.56ª	0.56ª	0.64ª	0.50 <sup>b</sup>	0.04
Protein efficiency ratio <sup>4</sup>	1.38 <sup>a</sup>	1.37 <sup>a</sup>	1.59 <sup>a</sup>	1.23 <sup>d</sup>	0.12

Means in the same row with different superscript letters are significantly different (P<0.05). S.E. standard error of the mean.

- 1- Specific growth rate = 100 X (Ln final weight- Ln initial weight)/ 120.
- 2- 2- Feed conversion= (feed given per fish)/ (weight gain per fish).
- 3- Feed efficiency= (weight gain per fish)/ (feed given per fish).
- 4- Protein efficiency ratio = (weight gain per fish)/ (protein intake).

As can be seen from Table (6), the feed intake was similar in the control and L10 diets. However, L20 aPnd L30 showed a decrease in feed intake. The slight tendency toward a lower feed intake as dietary lupin meal content increased could be due to a progressively more difficult adaptation of fish to the organoleptic properties of such diets. The same finding was recorded in salmo trout by De la Higuera et al., (1988). The decrease in feed intake of fish diets was increased where lupin level increased, probably due to the high content of fiber (L30) In the present study, the lupin used was a sweet variety with a lower content of alkaloids and it was extruded. The beneficial effects of extrusion treatment of row materials on their digestive utilization has already been reported (Kaushik, 1989 and Bangaula et al., 1993).

As presented in Table (6), the feed conversion, feed efficiency and protein efficiency ratio showed significant differences among treatments (p< 0.05). The best results were found with the fish fed at 20% incorporation of extruded lupin seed meal (L20). On the other hand, the least efficiency was recorded with 30% incorporation level (L30). These results were in agreement with the results of Gomes and kaushik (1989) and Bangaula et al. (1993).

Date of the apparent digestibility coefficient (ADC) of nutrients are showed in Table (7). The results showed no significant differences due to the incorporation of extruded Lupin up to 20%. However, the incorporation of 30% extruded lupin gave less digestibility coefficients than that of the control, L10 and L20, respectively concerning dry matter, protein and fat digestibilities. These results are in agreement with the results of Burel et al., (1998) in rainbow trout and Hassanen (1998) in Sparus aurata. As can be seen from Table (7), the results indicated good utilization of all extruded lupin tested, except that L30 level and the values were similar to those presented in the literature for fish meal and soybean meal (Pffeffer, 1982). The results of ADC (protein, lipid and carbohydrate) in the present trial were in agreement with the results of Yones (1989) in Sparus aurata. However, the less digestibility of carbohydrate in lupin diets may be a result of insufficient amylase enzymes in the intestine of Sparus aurata to metabolize dietary carbohydrate. Similar finding was recorded in salmon by Saponnhof and Plantikov (1983).

Difference in carcass composition, HSI and VSI between the control and the three incorporation levels were not statistically significant (Table 8). These results are in agreement with the results recorded for Sparus aurata by Pereira and Oliva-Teles. (2002).

Table (7) Apparent digestibility coefficients of the experimental diets.

		Die	ts		
Nutrients	Control	L,10	L20	L30	SE±
Dry mater	85.3ª	84.5ª	85.6ª	80.0 <sup>b</sup>	2.25
Protein	94.0ª	93.5ª	94.5ª	90.0 <sup>b</sup>	1.76
Fat	93.0 a	92.5ª	93.2ª	89.0ª	1.7
Carbohydrate	76.0ª	71.0°	72.0 <sup>b</sup>	68.0 <sup>d</sup>	2.86

Means in the same row with different superscript letters are significantly different (P<0.05)

Table (8), Final body composition, HSI and VSI for Sparus aurata fed the experimental diets (%dry mater basis)

		Diets				
Items	Initial	Control	L,10	L20	L30	
Drymatter	23.0	24.5ª	25.0°	25.2ª	25.4ª	
Protein	75.0	74.0ª	73.8ª	73.5ª	73.2ª	
Lipid	17.0	18.5ª	18.8ª	19.8ª	20.0ª	
HSI 1	108	2.4ª	2.2ª	5.5ª	2.8ª	
VSI <sup>2</sup>	2.1	4.3ª	4.5ª	4.8 <sup>a</sup>	4.6ª	

Means in the same raw with different super script letters are significantly different (P<0.05).

- 1-Hepatosomatic index = (liver weight/ body weight) × 100.
- Viscerasomatic index= (viscera weight/ body weight) ×100.

The results of the present trial suggest a maximum level of 20% incorporation from extruded lupin seed meal can be used in Sparus aurata diets. These results were in agreement with the results of Robaina et al., (1995) in the same species and within the maximum range (20- 40%) in trout diet which reported in previous studies (De la Higuera et al., 1988, Hughes,

1988, Gomes and Kaushik, 1989, Moyano et al., 1992, Bangaula et al., 1993 and Gauveia et al., 1993).

As presented in (Table, 9), the blood contents were not affected by the incorporation levels. Insignificant results were found among treatments. Similar results were recorded in *Sparus aurata* by Hassanen *et al.*, (1992), *Diplodus argenteus* (Filho *et al.*, 1992), coho salmon (*Oncrhynchus kisutch*) Higgs *et al.* (1978) and trout (*Salmo gairdeneri*) Alexis *et al.*, (1985). In the same trend, the plasma contents showed a significant differences among treatments. These results were in agreement with the results recorded in tilapia (*Oreochromis niloticus*) by Shemino *et al.* (1993).

Table (9).Hematocrite, hemoglobin and some plasme contents of

Baramatar	Diets				
Parameter	Control	L,10	L20	L30	
Hematocrite(%)	41.0 <sup>a</sup>	41.4ª	41.8ª	41.0 <sup>a</sup>	
Hemoglobin (g/100ml)	7.5ª	7.2ª	7.3ª	7.1ª	
Protein (g/100ml)	4.1 <sup>a</sup>	4.3 <sup>a</sup>	4.2ª	4.0 <sup>a</sup>	
Glucose (mg/100ml)	55.5ª	45.8ª	55.2ª	54.7ª	
Trigly ceride (mg/100ml)	242.0°	243.0°	242.5ª	241.6ª	
Phospholipid(mg/100ml)	576.0°	576.2ª	575.5°	574.5°	

Means in the same row with different superscript letters are significantly different (P<0.05).

In conclusion: the results of the present trial indicated that the partial replacement of fish meal by extruded lupin seed meal is very interesting in terms of growth performance. The main finding of this study is that extruded lupin seed meal up to 20% could beused, without adversely effect on growth performance, digestibility coefficient and blood characteristics of *Sparus aurata*.

#### REFERENCES

- Alexis, M.N. Papaparaskeva, E.and Teochaxi, V. (1985). Formulation of practical diets for rainbow trout (*Salmo gairdnari*) made by partial or complete substitution of fish meal by poultry by product and certain plant by product. Aquaculture, 50: 61-73.
- AOAC, (1995). Association of Official Analytical Chemists 14ed. Assoc Office, Anal. Chem, Washington, Dc.
- APHA, (1992). Standard Methods for the Examination of Water and Waste. Water American Public Health Association, Washington, DC, 1134pp.
- Bangaula, A.Parent, J.P. and Vellas, F. (1993). Nutritive value of white lupin (*Lupinus albus var lutop*) fed to rainbow trout (*Oncorhynchus mykiss*). Effects of extrusion cooking. Reprod. Nutr. Dev. 33: 325-334.
- Batterham, E.S., Andersen, L.H., Burnhan, B.V. and Toylor, G.A. (1986). Effect of heat on nutritional value of lupin (Lupinus angustilofius) seed meal for growing pigs. British Journal of Nutrition, 55.169-177.
- Blauth, D.J., M. Chareinski and H. Drelic (1963). Anew rapid method for determining tryptophan. Anal. Chem. 96-99.

- Bolin, D.W., King, R.P. and Klosterman, E.W. (1952). A simplified method for the determination of chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) when used as an index substance. Science, 116: 634-635.
- Burel, C.T. Baujarol, G. Corraze, Kaushik, S.T., Boeuf, G, Mol, KoA, Der Gayten, S.V. and Kuhn, E. R. (1998). Incorporation of high levels of extruded lupin diets for rainbow trout (Oncorhynchus mykiss) nutritional value and effect on thyroid status. Aququlture, 163, 325-345.
- Choubert, G. De la Noue, J. and Luquet, P. (1982). Digestibility in fish: improved device for the automatic collection of faeces. Aquaculture, 71:37-50.
- De la Higuera, M., Carcia- Gallego, M., Sanz, A. Cardenete, G. Suarez, M.D. and Moyano, F.J. (1988). Evaluation of lupin seed meal as an alternative protein source in feeding of rainbow trout (*Salmo gairdneri*). Aquaculture, 71: 37-50.
- Duncan, D.B. (1955). Multiple range and multiple F. test. Biometric, 11: 1-42.
- Filho, D.W., Eble, G.J., Kassner, G., Caprario, F.X., Dafre, A.L. and Ohira, M. (1992). Comparative hematology in marin fish. Comp. Biochem. Physiol. 10 A pp. 311-322.
- Folech, J., Lees, M. and Sloane Stanley, G. 1957. A simple method for the isolation and purification of total lipids form animal tissues, J. Biol. Chem. 226, 497-509.
- Gardner, W.S. and Miller, W.H. (1980). Reverse phase lipid chromatography of amino acids after reaction with opthaladehyde. Anal . Biochem., 101, 61-70.
- Gauveia, A., Oliva-Teles, A., Gomes, E. and p. Romer (1993). Effect of cooking/ expansion of three legume seeds on growth and food utilization by rainbow trout. In: Kaushik, S.J., Luquet, P.(Eds). Fish Nutrition in Practic, June, 24-27. Biarritz, France. INRA. Paris.
- Gladstones, J.S. (1970). Lupins as crop plants. Field Crop Abstracts 23, 123-
- Gomes, E.F. and Kaushik, S.J. (1989). Incorporation of lupin seed meal, colzapro or triticale as protein/ energy substitutes in rainbow trout diets. In. Takeda, M. and watanabe, T. (Eds). The current status of Fish Nutrition in Aquaqulture, Proceeding of the third International Symposium on Feeding and Nutrition in Fish. 28 August-1 September. toba, Japan, pp. 315-324.
- Hansen, R.P. and Zochanska, Z. (1974). Composition of the lipids of lupin seed. J.Sci Food Agric., 25: 409-415.
- Harding, J. and Hoglund, L,B. (1983). On accuracy in estimating fish blood variables. Comp. Biochem. Physiol., 75A: 35-40.
- Hassanen. G.D.I. (1998). Lupin seed meal compared with soybean meal as partial substitutes for fish meal in gilthead sea bream (*Sparus aurata*) diets. J. Agric. Sci. Mansoura Univ., 23: 141-154.
- Hassanen, G.D.I., Sheriff, Y.S., Daader, A.H. and Yones, A.M. (1992). Utiliztion of unconventional rations for feeding of gilthead sea bream (*Sparus aurata*). Egypt. J. Appl. Sci, 7: 566-586.

- Higgs, D.A, Market, J.R., Macquarrie, D.W., Mebride, J.R., Dosanjh, B.S, Nichols, C. and Hoaking G. (1978). Development of practical dry diets for coho salmon (*Oncorhynchus kisutch*) using poultry by product meal, feather meal, soybean meal and rapeseed meal as major protein sources (Cited from FAO/EIEAC symposium on fin fish nutrition and feed technology), EIFAC/78: E/43.
- Hill, G.D. (1977). The composition and nutritive value of lupin seed. Nutrition Abstracts and Reviews, B.: (Livestock Feeds and Ffeeding), 47:511-529.
- Hill, G.D.and Pastuszewska, B. (1993). Lupin alkaloids and their role in animal nutrition. In van der poel, A.F.B.Huisman,J., Saini, H.S. 9Eds). Second internaational workshop on Antinutritional factors (ANFs) in legume seeds. Recent Advances of Research in Antinutritional Factors in Legume seeds, 1-3 December wageningen Netherlands, EAAP Publication pp. 343-362.
- Hudson, B.J.F.(1979), Oualitas Plantarum- plant foods for Human. Nutrition, 29, 245- 251.
- Hughes, S.G. (1988). Assessment of lupin flour as diet ingredient for rainbow trout (*Salmo gairdneri*). Aquaculture 71:379- 385.
- Hughes, S.G. (1993). Use of lupin as a replacement for full- fat soy in diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 93: 57- 62.
- Jauncy, K. (1982b). The effect of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapia (Sarotherodon mossambicus). Aquaculture, 27: 43-54.
- Jauncy, K. (1988). A short guide to aquaculture feeds and feeding for Egypt. Published at university of Sterling, FKC, 4, LA, Scotland, U.K.
- Jobling, M.N. (1981). Dietary digestibility and the influence of food components on gastric evacuation in plaice (*Pleutonenectes platessal J.*). Fish Biol., 19: 29-36.
- Kaushik, S. G. (1989). Use of alternative protein source for intensive rearing of carnivorous fishes. In. Flos, R., Tort.L. and Torres, P. (Eds) Mediterranean Aquaculture. Ellis Hozwood. Chichester,pp. 125-138.
- Lopez- Bellido, L. and Fuentes, M. (1986). Lupin as an alternative source of protein. Ad Agron 40: 239- 295.
- Maynard, L.A. and Loosli, J.K. (1969). Animal Nutrition 6<sup>th</sup> edn Mc Graw Hill Book Company. London, 613pp.
- Moyano, E.J., Cordenete, G. and De la Higuera, M. (1992). Nutritive values of diets containing a high percentage of vegetable protein for trout (Oncorhynchus mykiss). Aquat. Living Resour, 5, 23-29.
- Pereira, T.G. and Oliva- Teles, A. (2002). Preliminary evaluation of pea seed meal in diets for gilthead sea bream (*Sparus aurata*) juveniles. Aquaculture Research, 33: 1183- 1189.
- Pffeffer, E. (1982). Utilization of dietary protein by salmonid fish. Comp. Biochem. Physiol., 73B (1): 51- 57.
- Porter, C.B., Krom, M.D. and Gordin, H. (1986). The effect of water quality on the growth of *Sparus aurata* in marine fish ponds. Aquaculture, 59:299-315.

- Priddis, C.R. (1983). Capillary gass chromatography of lupin alkaloids. J. Chromatogr., 26: 95-101.
- Robaina, L. Izquierdo, M.S. Moyano, F.J. Socorro, J., Vergara, J.M, Montero, D. and Fernandez-Palacios, H. (1995). Soybean and lupin seed meal as protein sources in diets for gilthead sea bream (*Sparus aurata*), nutritional and histological implication, Aquaculture, 130: 219-233.
- Roemer, P. (1983). *Lupinus alpus L.*, valuable future crop of old tradition. Grain, Legumes, 3: 14-17.
- Sabout, J.J. and P. Luquet (1973). Nutritional requirements of gilthead sea bream (*Chrysophrys aurata*) quantitative protein requirements. Marine Biology, 18:50-54.
- Saponnhof, L. and Plantikov, H.L. (1983). Studies on carbohydrate digestion in rainbow trout. Aquaculture, 30: 95-352.
- Shantha, N.C. and Ackman, R.G. (1990). Nervoric acid versus tricosanoic acid as internal standards in quantitative gas chromatographic analyses of fish oil longer chain n-3 polyunsaturated fatty acid methyl esters. J.Chromatogr., 533, 1-10.
- Shimeno, S., D. Ming and M. Takeda (1993). Metabolic response to dietary carbohydrate to lipid ration in *Orechromis niloticus*. Nippon Suisan Gakkaish, 59: 827-833.
- Shimeno, S., Hosokawa, H., Takeda, M., Takayama, S., Fukui, A. and H. Sosak: (1981). Adoption of hepatic enzymes to dietary lipid in young yellow tail. Nippon Suisan Gakkaishi, 47: 63- 69.
- Snedecore, W.G. and Cochran, W.C. (1987). Statical Methods. Iowa state Univ. 10. USA.
- Yones, A.M. (1989). Utilization of unconventional rations for feeding of gilthead sea brean (*Sparus aurata*). M.S. Thesis, Faculty of Agriculture, Zagazig University.

إدخال مسحوق الترمس كمصدر للبروتين النبائي في علائق أسماك الدنيس. عبد المنعم عبد الصادق مهدى يونس المعهد القومي لعلوم البحار والمصايد - محطة بحوث الأسماك بشكشوك الفيوم حصر.

تم استخدام مسحوق الترمس كمصدر للبروتين النباتي ليحل حزئيا محل مسحوق السمك في تغذية أسماك الدنيس حيث تم تكوين ٤ علائق تحتوى علي مسحوق الترمس بينما العلائية (٢٠,٠٠ عيل كانت العليقة المقارنة والتي لا تحتوى علي مسحوق الترمس بينما العلائية ما المعالمية من أصبعيات استخدام مسحوق الترمس فيها بنسب ١١٠ ٠٠ و ٣٠ علي التوالي. استخدمت ٢٠ أصبعية من أصبعيات الدنيس ذات الوزن الأولى (٩٤٥ عيل ١٠٠٠ عيث وزعت عشوائيا علي أحواض من الفيسرجلاس سعة الحوض ١م٣ وتم تغذية الأسماك بمعدل ٣٠ من وزن الجسم قدمت علي مرتين يوميا. فظهرت معدلات أداء النمو نتائج معنوية علي مستوى (١٠٠٠ العلقة المقارنة والعليقة المحتوية على ١٠٠ محدلات أداء منخفضة لم يتاثر تركيب جسم الأسماك بمستوى مسحوق الترمس المستخدم في العليقة وكذلك صفات الدم من الهيماتوكريت، الهيموجلوبين ومحدوى البلازماء وأظهرت معاملات الهضم للعلائق استفادة جيدة لمسحوق الترمس المستخدم في العلائق وخاصة معاملات الهضم المستخدم في العلائق معاملات الهضم المستخدم في العلائق خاصة على ١٠٠ من الهيماتوكريت، الهيموجلوبين خاصة على أداء الأسماك بمستوى ١٠٠ من العليقة بدون تاثيرات خاصة على أداء الأسماك الدنس.