Effect of feeding levels on Carcass and Liver of Gilthead seabream, *Sparus aurata* (Linnaeus, 1895)

Nasser K El-kebir (*) & Ali R. Mujahid

Dept. of Zoology, Faculty of Science, University of Zawia

Abstract

The experiments were performed on (Gilthead seabream, Sparus aurata L.) , which is one of the important species to the aquaculture industry in the Mediterranean region. A feeding experiments was conducted to investigate the influence of feeding levels on the carcass and liver of S. aurata. Four feeding levels were employed: 1.0% ; 2.0% ; 3.0% and 4.0%BW/day. The lipid content of carcass and liver show an increase with an increasing of feeding regimes. Important changes were observed in the final carcass and liver in terms of lipid content

(*) Email: n.alkabeer@zu.edu.ly

comparing to the initial. Histological examination showed an increase of lipid deposition around the liver cells and blood vessels due to an increase of feeding levels. Hepatosomatic index (HSI) and condition factor (K) showed the same trend, high in fish fed higher feeding levels and lower in fish fed low feeding regimes. It is probably due to many factors one of these the high lipid content in the experimental diet. The studies so far provide evidence that the feeding levels is one of the main factors influencing the carcass and liver proximate composition of the fish. These results could be have an important implication in terms of industrial diet, fish quality, and it's economic as well as human health.

Keywords: *Feeding levels; carcass; liver; Sparus aurata.*

1. Introduction

The gilthead seabream, *Sparus aurata* (*L.*), (order : Perciformes, Family: Sparidae), is a marine temperate water fish species which has eurythermal (10-36 c^{o}), and euryhaline (5-60 ppt) capacities (FAO, 2000, Coll, 1983; Chervinski, 1994); and it is resistant to high variation in the environment. *S. aurata* is abundant in the Mediterranean Sea and has also reported in the black sea and eastern Atlantic coasts (FAO, 2002 ; UNESCO, 1995). *S. aurata* was first cultured in the early 1970's in intensive system in Italy with other country joining this effort in later year (Kissil,1999). *S.aurata* have become very important in the Mediterranean region distinguished by their quality market value (Anon,1993). *S.aurata* is cultured and grown in different farming system both extensively, in brackish water lagoon, and intensively, in tanks, ponds and floating cages.

Production of finfish from marine aquaculture has been grown steadily during the past decade. According to Autin (1997), the production of *S.aurata* and *Dicentrarchus labrax (L)* in the Mediterranean has increased dramatically in recent years from(30,000 metric tons in 2000 to 40,000 mt in 2010 to 48,500 mt in 2011 and to 55,000 mt in 2015). By the end of 2016 the figures expected a much higher than those in the previous year FAO (2015). In fish farming of *S.aurata* and other fin fishes such as *D.labrax*, both the lipid composition and quality of feed have an effect on body composition ; fatty acid pattern of the fillet as well as the liver of the fish. (FAO, 2005; Johansson & Kiessling, 1991 ; Alexis et al, 1995 and Messina et al, 2013). Farming *S. aurata* have a higher fillet lipids content than the wild fishes , and different fatty acid pattern, this is due first and foremost to the composition of their diet and also to the increase of feeding regimes during the farm operation. Involving regulation of the feed ratio have been studied by (Asgard et al, 1988; Huisman, 1996 ; Reinitz, 1998 and Wang, et al, 2014).The feeding levels and feeding time of the fish farming such as *A.salmon and S.aurata*, is one of the main factors influencing growth, body composition, liver cells, as well as fish quality .(Storebakken & Austreng, 1997; Montoya et al, 2010 and Martha et al, 2015). The aim of the studies is to carry out to investigate the effect of feed levels on carcass and liver of Gilthead seabream (*Sparus aurat*a. *L*.).

2. Materials and Methods

2.1. Experimental set-up

The experiments were carried out over a period of 15 weeks. This corresponded to a total of (105) days including an acclimation periods of (3) weeks.

2.2. Experimental tanks

Twelve square white fiber glass tanks (1.5 m^3) located indoor were used , an open system was used during the experimental period. The tanks received a borehole water of constant salinity of (37%), and water temperature of (20 ^{0} c). The water was supplied at a rate of between 20-25 L/min for each tank to maintain an adequate dissolved oxygen level at excess of 8.0 mg/L and a constant temperature of (21 $^\circ$c).

2.3. Proximate analysis of fish and feed

360 specimens of Gilthead seabream, *Sparus aurata*, were obtained from the graded stock of a private fish farm (Farwa Aquaculture Project), and transfer to the experimental tanks of Marine Biology Research Center in Tajura, with sufficient oxygenation. The experimental fishes had an average body weight of 110 grams , the fish was distributed randomly in the tanks (30 fish /tank).At the beginning and at the end of the experiment, after being starved for 24 hours , a random sample of 10 specimens from each tank was collected for the carcass composition . The fishes collected for carcass analysis were frozen at -20 ^{0} C before homogenized for proximate analysis. A pelleted commercial food of 2.0 mm diameters (PROVIMI. Marine Grower E: Holland), the proximate analysis of food composition used in the experiment is given in Table.1. Four feeding levels were employed over an experimental period of 12 weeks. The tanks were randomly assigned to the following treatments: (1.0%, 2%, 3.0% and 4.0%). Proximate analysis of carcass and feed were performed according to standard procedure (ISO, 2000 and AOAC, 2002).

2.4. Histology examination

Fish samples were taken for histological examination of the liver at the start and at the end of the experiment. Standard techniques of histology were used (Stevens and Wilson, 1998 and Cook, 1996). Five fishes from each tank (i.e-10 from each treatment) were selected randomly at the beginning and at the end of the experiment. The fish were killed by pitching. They were then opened and their liver taken out and put in labelled containers with fixative. The samples of liver were stored at 20 ^{0}C until analysis.

2.5. Hepatosomatic index and Condition factor

At the beginning of the experiment, after being starved for 24 hours, a random sample of 5 fishes from each tank, corresponding to 30 specimens from all treatments, was collected for the determination of hepatosomatic index (HSI) and condition factor (K). At the end of the experiment, again after starving, a random sample of 10 fish was collected from each tank for the same determination. The fish were killed by an overdose of anesthetic (2-phenoxyethanol at 0.6mg/L). The fresh fish were individually measured (fork length in centimeters ; body weight in grams) and the liver were weighed individually on an electronic balance with an accuracy of 0.1 mg.

3. Statistical analysis

Using the individual data for the three replicates of each treatments, statistical analysis was carried out to establish whether there were any significant differences between different treatments. The Student-Newman-Keuls Multiple Range Test (Zar, 2001) was used at a level of significance of $p<0.05$. HSI and K- factor were calculated using the following formula [HSI = liver weight / fish weight x 100] and [K = fish weight / (fork length)^3 x 100] (Nelson and Johnson,2001).The analysis was performed on a personal computer using BMDP Statistical Software Package (Version PC 90).

Table.1. Proximate composition (%) of the experimental diet (PROVIMI. Marine Grower E; Holland).

Proximate Composition (%)	Crude protein	Crude lipid	Crude fiber	Moisture	Ash
	47.00	13.11	2.71	7.94	9.50

Proximate analysis (% wet weight).

4. Results

4.1. Assessment of proximate composition

The proximate composition of the fish carcass at the start and at the end of the experiment are shown in (Tabe.2). In general the body composition was greatly affected by the different feeding levels, but some interesting changes were noticed. There was a higher value (18.33) in final carcass lipid in fish fed feeding level of 3.0% and a lower value (12.22) in fish fed feeding level of 1.0% compared to the initial. The final analysis of protein, Moisture , ash and phosphorus contents did not vary from initial for all feeding regimes.

Tabe.2. Initial and final proximate composition of carcass of *Sparus aurata* fed different feeding levels.

Carcass	Initial	Feeding levels (%)			
		1.0	2.0	3.0	4.0
Moisture	61.14	59.98	60.44	61.67	62.33
Crude protein	17.12	18.22	19.33	20.15	20.25
Crude lipid	15.86	12.22	14.82	18.33	17.88
Ash	03.11	03.21	03.54	04.22	03.99
Phosphorus	01.15	01.10	01.13	01.14	01.12

Proximate analysis (% wet weight).

4.2. Assessment of liver histology

Histological examination of liver sections (Plates. 1 and 2) showed an increase of lipid content of the liver with an increasing of feeding levels . Lipid content was higher in 3.0% and 4.0% feeding levels, and lower in feeding levels of 1.0% and 2.0%., considerable lipid level was deposited in the hepatocytes as compared with the specimens at the start of the experiment. In contrast minor changes was noticed in those fish fed low feeding regimes.

4.3. Assessment of Hepatosomatic index

The results of initial and final hepatosomatic index (HSI) of *S. aurata* during the experimental period are shown in Table (3). The final hepatosomatic index showed higher among different feeding treatments. Feeding levels of 3.0% and 4.0% showed some differences from other feeding regimes. The final values of (HSI) ranged between (1.62 - 2.44) in feeding levels of 1.0% and 4.0% respectively.

Table.3. Initial and final hepatosomatic index (HSI) of *Sparus aurata* fed different feeding levels

	Feeding levels (%)			
	1.0	2.0	3.0	4.0
Initial (HIS)	1.26^{nd} (0.0)	1.20^{nd} (0.0)	1.22^{nd} (0.0)	1.25^{nd} (0.0)
Final (HSI)	1.62^{a} (0.1)	1.66^{a} (0.2)	2.15^{a} (0.2)	2.44^{a} (0.3)

Means in a row followed by the same superscript are not significantly different ($p<0.05$)

Numbers between parameters refer to standard deviation

nd = not determined

4.4. Assessment of Condition factor

Condition factor (K) or coefficient of condition, as a fatness index of *S. aurata* fed on different feeding levels are shown in Table (4). Initial (K) showed the highest value (1.61) . The final results of (K) showed some variation between different feeding regimes, highest in feeding level of 4.0% , followed by 3.0% and lowest in feeding level of 1.0% respectively. The final (K) values ranged between 1.44–2.21 in feeding levels 2.0% and 4.0% respectively.

Table.4. Initial and final condition factor (K) of *Sparus aurata* fed different feeding levels.

	Feeding levels (%)			
	1.0	2.0	3.0	4.0
Initial (K)	1.52[nd] (0.1)	1.30[nd] (0.1)	1.61[nd] (0.1)	1.35[nd] (0.1)
Final (K)	1.54[a] (0.1)	1.44[a] (0.1)	1.84[b] (0.2)	2.21[b] (0.3)
Mortality (%)	1.11	0.00	0.00	0.00

Means in a row followed by the same superscript are not significantly different ($p<0.05$)

Numbers between parameters refer to standard deviation

nd = not determined

4.5. Mortality

Minor mortalities have been recorded during the experimental period. Mortalities were two fishes (01.11% mortality) in feeding level of 1.0%. These value are considered as normal for *Sparus aurata* (*L.*).

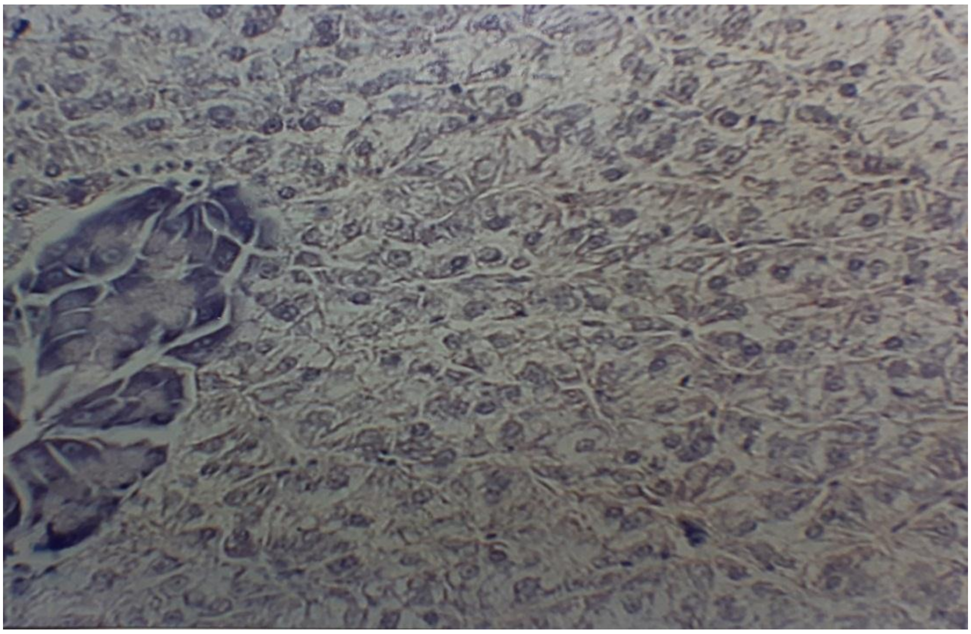
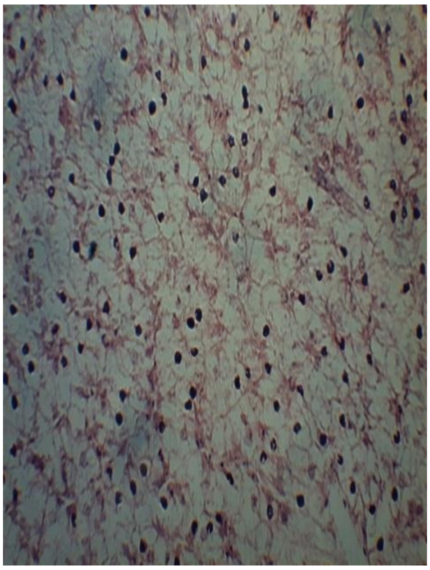
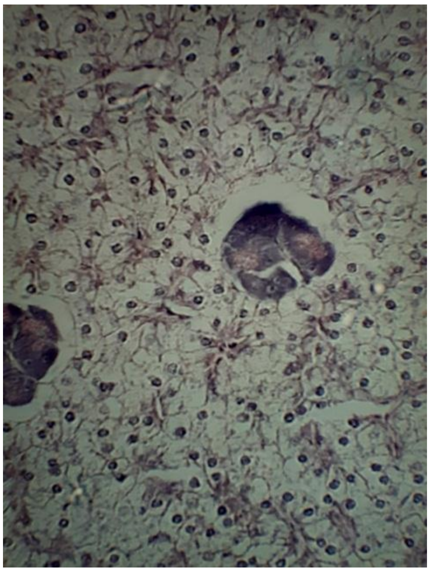
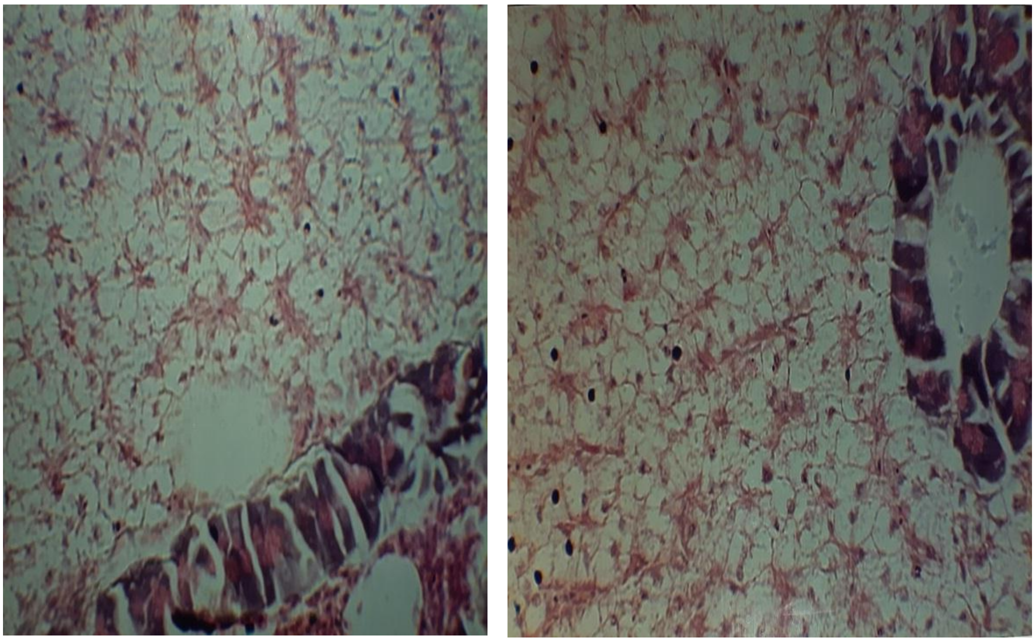


Plate.1. Liver section of *Sparus aurata*, at the start of the experiments





(A) **(B)**



(C) **(D)**

Plate.2. Liver sections of *Sparus aurata* , at the end of the experiment, from fish fed different feeding levels. A(1.0%); B(2.0%); C(3.0%) and D(4.0%).

5. Discussion

From the results of this experiments, differences were clearly obvious between the feeding levels. The proximate composition of the whole fish carcass was greatly affected by the different feeding level . The final carcass lipid showed increased in feeding levels at 3.0% and 4.0% and decreased in fish fed 1.0% and 2.0% feeding level compared to the initial (Table.2) . Lipid in fish carcass increase with an increase of feeding regimes. Shearer (1994) found differences in proximate composition of fish carcass due to many factors such as size of fish, food composition, energy intake. lipid content tends to increase with an increase of feeding levels. Umino et al (1991) working with the similar species, where muscular lipid content and intraperitoneal fat bodies increased with growth of fish, also (Martha et al, 2015 and Messina et al, 2013) found increased lipid ratio and hepatic cells of the catfish with increasing of fish size and diet. As in most other species, moisture, protein, ash and phosphorus contents were observed to be more or less stable components of *S. aurata* carcass. Love (2004) has reported that lipid is the most variable component in fish carcass and liver. In the present investigation an increasing of lipid content in fish carcass due to increasing of feeding levels from 3.0% to 4.0% , resulting in an increase of lipid content in some organs of the fish such as liver or abdominal cavity of fish. This results conform to the results reported by Hasan and Macintosh (1993) for *C. carpio* (L.) feeding at a level of 3.0% gave a higher carcass lipid with an increase of feeding. In the present study the fish were grown at a temperature between 20^0C to 22^0 C and fed a levels between 1.0% to 4.0% . It appears clear from the analysis of the experimental diet (PRVIMI. Marine Grower), used during the experimental period that the protein content (47.00%) , is sufficient to meet the protein requirement of *S. aurata*. This is supported by the results reported by many authors, such as Kissil et al (2001) and Vergara (1992). This was believed to be the result of higher feeding levels as well as increase lipid content in feed experiment. From the final sections of the liver (Plate.1 & 2), show a significant accumulation of fat droplets deposited in the hepatocytes of the fish fed higher feeding levels compared to the initial sections , this is probably due to the fact that the industrial marine fish diets still need improving in the case of *S. aurata*. The observation from the histological examination of liver sections conform to the observation by (Paloheimo and Dickie, 1996 ; Vasquez et al, 2013 and Wang et al, 2014). In contrast , lipid content undergoes wide variation particularly in adult fish. According to the study reported by (

Marais and Kissil, 2001 ; Metailler et al, 2004). The increase in lipid content of fish carcass and liver is a frequently observed phenomenon. Hepatosomatic index (HSI), it gives an estimation between the weight of the liver and the weight of the fish. It is also linked with the fat deposition of liver. At the end of the experiment, HSI was higher in fish fed at a feeding level 3.0% and 4.0% and lower at feeding levels 1.0% and 2.0% compared to the initial values. Umino et al (1991) found the same results with *Pagrus major*, where the lipid deposition increased with an increasing of feeding levels and growing of fish. Martha et al (2015) and Wang et al (2014), working on Catfish, found increased of lipid in hepatic histology of body composition due to the effect of feeding and increasing of fats in commercial food.

The condition factor (K), or coefficient of condition, as introduced by Nilson and Johnson (2001), measures the health state of fish. It is often linked with the fatness state of the fish , that is, it gives an estimation and relationship between the length and weight of the fish. (K) values showed the same trend seen in the hepatosomatic index. The final (K) increased in fish fed higher feeding levels and decreased in fish fed lower feeding regimes. As a matter of fact, there is a considerable number of variables that can affect the value of K such as biological, environmental and underfeeding, etc. The final condition factor obtained in this experimental clearly showed consistence change in health status of fish compared to controls (Table.4).

In conclusion, it is evident that the effect of feeding levels on carcass and liver has to be studied from two different subjects. The first is that of economy , which is determined according to the fish farming program, and the other is related to the physiological response. From the economic point of view, it is difficult to determine the optimum feeding level , as the production plan of fish farming and there are a number of marketing factors that effect it. From the physiological point of view, the fish utilization off commercial food as a whole, taking in consideration all factors which affect it, such as fish size , digestion, fish biorhythm, quality as well as food wastage. Although, according to the previous results reported that, increasing of feeding regimes will permit an increase of lipid content of carcass and liver. Feeding of 3.0% and 4.0% considered a high feeding levels for *S. aurata*, taking in consideration the economic factors due to many considerations during the feed management of fish farming in order to decrease the feed level and production costs.

6. Conclusion

In conclusion, the results from the present investigation provide evidence to show that feeding levels can influence the carcass and liver lipid of Gilthead seabream, *Sparus aurata*. The high lipid content in proximate composition and the histology examination of liver were as observed when the fish fed higher feeding regimes. Final results of Hepatosomatic index and condition factor showed the same trend, an increase with an increasing of feeding levels and decrease in fish fed lower feeding levels. The result indicated that feeding levels is one of the factors effecting the whole-body composition and quality of the fishes. Increasing of lipids in carcass composition and significant accumulation of lipids and fat droplets deposition in the hepatocytes of fish liver is probably due to the fact that the commercial food industry of *S. aurata* still need improving in terms of lipid contents as one of the factor of quality. These conclusions could have a very important implication in commercial food industry, fish quality and it's economic as well as human health.

Acknowledgements

The authors would like to acknowledge, all the staff of MBRC especially the staff for their assistance and helping us to sort out the technical problems of the experimental system and water supplies during the actual running of the experiments. A special thanks goes to the Director of the MBRC library for providing us with some references.

References

1. *Asgard. T. Storebakken. T; Austreng. E & Vabo. P.H. (1988). Ratio level for salmonds – influence on growth, feed conversion and body composition. Abstr. Int. Symp.. Feeding and Nutrition of Fish. Bergen : pp 23-27.*
2. *Alexis. M.A;Theochari. V. & Papaparaskeva-Papoutsoglou. E.G. (1995). Effect of diet composition and protein level on, body composition, haeatological characterestics and cost of production of Rainbow trout. Aquaculture. 58 : pp 75-85*
3. *Anon, M. (1993). Market trends. INFOFISH International. No.1/93. Jan/Feb.37pp.*
4. *AOAC (2002). Official methods of analysis of association of official of analytical chemists . Vols I and II, 15th ed. Washington DC. 1298 pp.*
5. *Autin, M. (1997). Commercial aquafeed manfacture and production. A review. Cahiers Options Mediterranean, 22, Feeding Tomorrow's Fish, CIHEAM : pp 79-105.*
6. *Chervinski, J.(1994).Salinity tolerance of young gilthead seabream (Sparus aurata L.). Bamidgeh. Vol.36, No.4, pp120-125.*
7. *Coll, M.I.(1983).Fabricacion de Pienson. In: Aquaculture Marine Animal. Madrid. Ediciones Mundi Prensa, pp430-446.*

8. *Cook, H. C. (1996). Carbohydrates. In : Theory and Practice of Histology Techniques. (J.D. Bancroft and A. Stevens eds.). Churchill Livingstone : pp 173 – 211.*
9. *FAO (2000).Development of coastal aquaculture in the Mediterranean region ADCP/MR/79/5.FAO, 1979. 69pp.*
10. *FAO (2002). Fish species identification sheets for fishery purposes, Mediterranean and Black sea. Vol. I & II : pp 22-30.*
11. *FAO (2005). Feed and feeding of fish and shrimp. ADCP.REP. 26 : pp 18-125.*
12. *FAO (2015). Planning and management for sustainable coastal aquaculture development.Rep.Stud.GESAMP, 68 : pp 56-63.*
13. *Hasam, M.S. and Machintoch, D.j. (1993). Effect of environmental temperature and feeding rates on growth, food utilization and body composition of (Cyprinus carpio),fry fish nutrition practice. Journal of Fish Nutrition (61) : pp 767-778.*
14. *Huisman.E.A. (1996).Food conversion efficiencies at maintenance and production levels for Carp. Cyprinus carpio , and Rainbow trout. Salmo gairdneri. Aquaculture. 9 :259-276.*
15. *ISO (2000).International Organization for Standardisation. Animal feeding stuffs-determination of moisture content. Ref.No. ISO. 6496-1983 E.*
16. *Johansson. L; kessling.A & Carlsson. R. (1991). Eating quality and growth of Rainbow trout on feed with different admixtures of leaf nutrient concentrate. Jor. Sci. Food Agri.. 57 : pp 217-234.*

17. Kissil, G.H.(1999). Known nutritional requirements of the gilthead seabream (Sparus aurata L.) in culture. European Maricult. Soc. Spec. Publ. 6, pp 49-55.

18. Kissil, G. H; Meyers, S.P; Stickney, R. R. and Gropp, J. (2001). Protein ratio in the feed of the (Sparus aurata L.). Proc. Warm water fish culture workshop. Baton Rouge, L.A. World Mariculture Soc. Spec. Publ; No 3, pp 145-152.

19. Love, R.M. (2004).The chemical biology of fishes. Academic Press, London: pp 255 -262.

20. Marais, J.K. and Kissil, G.W. (2001).The influence of energy level on the feed intake, growth, food conversion and body composition of Sparus aurata. Aquaculture. 17 : pp 203 – 219.

21. Martha. J, Redrigo. S, Leando. C, and Priscila. R (2015). Effect of fixed feeding time on growth , body composition and hepatic histology of Hybrid catfish (P. reticulatus X L. marmoratus). Fed with carbohydrates and lipids ratios. Medellin. Jor. ; 28 : pp 83-92.

22. Messina. A, Piccolo. G, Tulli. F, Cardinalettli. G (2013).Lipid composition and metabolism of European seabass (Dicentraruchus labrax. L), fed diets containing wheat gluten and legume meals as substitutions for fish meal. Jor.Aqua; 8 : pp 376-379.

23. Metailler, R; Aldrn, J.F; Messager, J.L; Mevel, G. and Stephan, G. (2004). Feeding of European seabass (Dicentrarchus labrax L.).Role of protein level and energy sources. Jor. World Maricul. Soci. 12(2) : pp 117 – 118.

24. *Montoya.A; Lopez. O; Yufera. M; and Sanchez. V. (2010).Feeding time , daily rhythms of behavior and digestive physiology in Glithead seabream (Sparus aurata. L.).Jor.Aqua; 306 : pp 315-321.*
25. *Miglavs, I. and Jobling, M. (1989). Effects of feeding regimes on food consumption, growth rates and tissue nucleic acids in juvenile Arctic chart, Salvelinus alpinus, with particular respect to compensatory growth. J. Fish. Biol. 34 : pp 947-957.*
26. *Nielson L. A and Johnson D.L. (2001). Fisheries techniques. American Fishery Society. Bethesda. Md : 468pp.*
27. *Paloheimo , J. E. and Dickie, L.M. (1996). Food and growth of fishes. II. Effects of food and temperature on the relation between metabolism and body weight. Jor. Fish. Res. Board. Can. 12 : pp 869 – 908.*
28. *Reinitz. G. (1998). Influence of diet and feeding rate on the performance and production costs of Rainbow trout. Trans. Am. Fish. Soci. 112 : pp 830-833.*
29. *Shearer, K.D. (1994). Factors affecting the proximate composition of cultured fishes with emphasis on Salmonids. Aquaculture. 119 : pp 63 – 88.*
30. *Stevens , A. and Wilson, I. (1998). The haematoxylin and Eusin. In : Theory and practice of Histological Techniques. (J.D. Bancroft and A. Stevens eds.). Chureill Livingstone : pp 99 – 121.*
31. *Storebakken. T. & Austreng. E. (1997).Ration level for Salmonids Growth survival, body composition, and feed conversion in Atlantic Salmon and S. aurata. Aquaculture. 60 : pp 189 – 206.*

32. Umino, T ; Nakagewa, H. and Takaba, M. (1991). Lipid accumulation and starvation tolerance in Red sea bream (Pagrus major). Nippon Suisan Gakkaishi. 1991. Vol.57. No.10.

33. UNESCO (1995) Check-list of fishes of the North eastern Atlantic and of Mediterranean (Biology and ecology). I & II : pp 120 -165.

34. Vasquez-Torres. W and Arias-castellanos. A. (2013). Effect of dietary carbohydrates and lipids on growth of Cachama fish (P.brachypomus). Jor. Aqua. Res; 44 : pp 1768-1776.

35. Vergara, J.M. (1992). Studies on the utilization of dietary protein and energy by gilthead seabream (Sparus aurata L.). Thesis University of Stirling. 164pp.

36. Wang. L, Liu. W, Zhang. C and Qian. Y (2014).Effect of dietary carbohydrates and ipid ratios on liver histology of Yellow cat fish (P.fulvidraco). Jor. Aqua; 426 : pp 41-48.

37. Zar, J.H. (2001). Biostatistical analysis (3rd Edition). Englewood Cliffs, NJ. Prentice-Hall.