Feeding European Sea Bass (*Dicentrarchus labrax*) With Trash Fish
1- Growth Performance and Reproductive Histology

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Abstract: The **effects** of exposing commercial feeds for European sea **bass** (*Dicentrarchus labrax)* were examined to lower prices diet. Trash fish used as aqua feed were submitted to six feeding schedules in net pen cultivation (40 fish/net pen)**. Each** treatment had two replicate net pen. The present study was performed to investigate the influence of individual fed local trash fish (8.6% of body weight) or dry pellet diet (2.2% of body weight) with four feeding schedules (Fs) as fed European sea bass (**a** range initial body weight **from** 19.57 to 24.85g) for 140 days. Fs1: 1.1% of body weight (BW) dry pellet at 9.00am and 4.3% of BW. **Local** trash fish at 14.00pm; Fs2: one day 2.2% of BW. Dry pellet diet consecutive another day 8.6% of BW. Local trash fish; Fs3: one day 2.2% of BW Consecutive two days 8.6% of BW Local trash fish; Fs4: two days 2.2% of BW. Dry pellet diet consecutive one day 8.6% of BW local trash fish. Growth performance, survival rate, proximate analysis of dorsal **musculature** and some somatic parameters of European sea bass were **measured** to assess four feeding schedules compared to fish fed only local trash fish or dry pellet diet. The improved of weight gain percentage, geometric mean were obtained in fish fed with feeding schedule No. 4(2 day fed dry pellet diet with one day feed local trash fish) compared to other feeding schedules. The survival rate of *D. labrax* feeding schedule No. 4were higher compared to other fish feeding schedule 1, 2, 3. Proximate analysis of dorsal **musculature** of different feeding **schedules showed** significantly ($P <$ 0.05) concentrations of lipid compared to fish fed only local trash fish as dry pellet diet. Small, round and transparent oocytes with a central nucleus were observed in histological sections of ovaries. Though, testes based on microscopic observation **were** distinguished for male *D.**labrax*** and **spermatogonia** and primary spermatocytes were the dominant cells of this stage. The results of this study **revealed** that **the** overlap between local trash fish with dry pellet diet under feeding schedule No.4, could be used in fed European sea bass (*D. labrax*) to give better growth performance.

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1. Introduction:

Economically productive aquaculture systems depend upon an adequate supply of low-cost feeds with high nutritional quality. Feed is considered as the most expensive single factor since feed cost constitutes at least 50% of the total production **(Posadas, 1988)**.

Trash fish, as commonly defined is that portion of the catch that by virtue of their small size or low consumer preference has little or no value (**Sugiyama *et al*., 2004)**. The same authors, showed that the use of term trash fish **varies** from country to country and can change both seasonally and with locations. **Xu *et* al. (2007) mentioned** that trash fish, mainly from capture fisheries, are not consumed by human due to their low protein content and relative un palatability. Trash fish are often discarded as by-catch, with potential environmental and aesthetical problems **(Li *et al*., 2004)**. In aquaculture**,** trash fish fed directly to carnivorous fish or as an ingredient of artificial feed are considered better alternatives **(Xu *et al*., 2007)**.

Bunlipatanon *et al*. (2004) reported that better cost-benefit and resource use, to fed Asian sea bass (*Lates calcarifer*) and tiger grouper (*Epinephelus fuscoguttatus*) were recorded for fish reared on trash fish. **Hung and Mao (2010) studied the** effect of different trash fish with alginate binding on growth and body composition of juvenile cobia (*Rachycentron conadum*). They ground raw fish and extruded by an extruder. Sodium alginate was used as a binder for all moist diets at a concentration of 3%. After extrusion, the moist diets were submersed in 10% $CaCl_2$ solution to gel through the strong binding of calcium and alginate for 10 minutes. Feeds were sealed in vacuum packed bags and stored frozen (-20°C) w\until feeding. **Peres and Oliva-Teles, (1999)** found that the optimum protein level in the diets for sea bass juveniles was estimated to be around 50%.

An important number of studies has been developed on the relations between nutrition and reproduction in several fish farmed species and some effort has been paid on the development of fish farming **(Asturiano *et al*., 2006)**. In several studies have shown that n-3 series enriched diets have profound effects on female reproduction, influencing pattern of gonadal development, plasma levels of lipids and sex steroids, egg quality and lipid levels, fecundity, hatching and survival rates of European sea bass **(Asturiano *et al*., 2006)**. Though, **Asturiano *et al*. (2001) described** the influence of series n-6 PUFAs on reproduction of teleost fish. **Chaitanawisuti *et al*. (2011)** fed the broodstock spotted Babylon (*Babylonia aerolata*) by trash fish once daily at 10:00h with the daily amount calculated as 15% of the total broodstock biomass per tank (excess diet was removed and feeding rate was adjusted based on weight gain after each sampling, which was done every (2 weeks).The mechanism linking gonad development and reproductive performance in order to improve culture techniques **(Cek and Yilmaz, 2007)**. The same authors showed that one of the most important factors necessary in the successful culturing of a fish species is obtaining a basic understanding of its key biological processes. The most important of these biological processes is the fish's reproductive cycle and formation of gametes.

A number of reports have demonstrated that gonad development in fishes is highly sensitive to exposure to substances with oestrogenic activity **(Maack and Segner, 2003)**. The structural alterations induced by exposure to environmental oestrogens can include the development of ovo-testes, the manifestation of gonadal malformations and histopathological alterations, or the increased frequency of atretic oocytes **(Nolan *et al*., 2001)**. **Mahmoud (2009)** reported that a **thorough** study of gonad anatomy and histology is required for proper management of the fishery.

The present work tries to define the course of local trash fish and dry pellet diet as fed in European sea bass (*Dicentrarchus labrax*) according to changes in four feeding schedules. This is considered in association to **lowering** the costs of feeding and the environmental risks.

2. Materials and Methods

Experimental fish and rearing condition

The experiment was carried out in twelve (12) net pen culture (diameter 4.0m, depth water 1.2m.) inside commercial farm (earthen pond) at the **Port Said** governorate, Egypt. Juvenile European sea bass (*Dicentranchus labrax*) were obtained from a local commercial fish farm 480 homogeneous fish with **a** range **of** initial body weight **from** 24.85 to 19.57g and distributed in 12 net pen at 40 fish per net pen. Fifteen-days acclimated to the commencement of the experimental period. During the acclimation period fish were gradually fed the dry pellet diet by starving them, followed by feeding local trash fish plus dry pellets, then progressively reducing the trash fish until dry pellets were completely accepted as has been by **Stirling (1977).**Fish **were** hand fed in plastic box. The feeding trials were conducted for 140 days (from June to October,2012).

Experimental diets and feeding schedule

The **experiment** was set up a six different diets fed in duplicate. The experimental diets consisted of one pelleted diet and one local trash fish with four different feeding schedule. The feeding rate from dry pellet diet was 2.2% of body weight and to compensate feeding rate from local trash fish 8.6% of body weight (for the higher water content), respectively. Daily ration were fed twice a day (equal meal at 9.00 am and 14.00pm h) according to feeding rates. Two test diets contained dry pellet diet and local trash fish, whilst in four feeding schedule were as follows:

Feeding schedule one (FS_1) was daily ration fed twice (1.1% of body weight dry pellet diet at 9.00am and 4.3% of body weight local trash fish at 14.00pm).

Feeding schedule two (FS_2) was daily ration, one day 2.2% of body weight dry pellet diet and another day 8.6% of body weight local trash fish.

Feeding schedule three (FS_3) was ration one day 2.2% of body weight, dry pellet diet and follow two day 8.6% of body weight /day, of local trash fish.

Feeding schedule four (FS4) was ration two day 2.2% of body weight dry pellet diet and follow one day 8.6% of body weight local trash fish.

Diet formulation and trash fish preparation

Local trash fish were collected from **fishermen** of **Lake** Manzala (belonging to Port Said Governorate, Northern Egypt). Local trash fish were frozen in a cold storage at – 10C° respectively. Before feeding, carcasses of frozen fish were minced by meat grinder into pieces and thawed for two hour respectively before feeding. Ingredients diet (obtained from a local feed company) Proximate composition and formulation (Table 1,2). Proximate composition of the local trash fish and formulate diet are shown in Table (3). All **ingredients** were **exposed to** grinding, mixture and processed into a California pellet Meal machine (CPM) as dry pelleted diet.

Growth trial

At the beginning of growth trial the feeding rate was adjusted based on weight gain after each sampling, which was done every 28 day.

A representative sample of ten fish was withdrawn and kept frozen (-4C°). Growth trial was conducted for 20 weeks (June-October) and every fourth weeks fish in each net pen were bulk-weight and counted to follow, growth and feed intake. At the end of the growth study and after an overnight fast, all fish from each net pen were individually weighed, total length measured and calculated to determine survival rate (%). Though fish from each net pen were randomly sacrificed and pooled for dorsal **musculature** composition analysis, weighed liver, and gonad (ovary, testis) for **determining** somatic-index and **examined** histological **histologically** for gonad **and** intestine.

Chemical analysis

Samples of feed ingredients diets and fish muscle were analyzed in triplicate using standard methods **(AOAC, 1997).** Dry matter was determined by drying in an oven at 105C° for 24 h. Nitrogen (N) was determined by the kjeldahl method and crude protein (CP) was calculated as NX 6.25. Crude fat (EE) content was analyzed using the soxhlet method with petroleum ether (bp 40 to 60C°) crude fiber (CF) content was determined by standard method **(AOAC, 2000).** Ash content was determined by incineration in a muffle furnace at 550C° for 12 h. Gross energy was calculated by **Martinez – Liorens *et al.* (2007)** as energy coefficients: protein 23.9kj/g, lipid 39.8kj/g and carbohydrate 17.6kj/g. Nitrosen free extract (NFE) was computed by taking the sum values for crude protein, lipid, ash, fiber and moisture, and subtracting this from 100.

Gonads were preserved after dissection in 10% formalin, dated and labeled with all the necessary data for subsequent examination. Paraffin embedded samples of the ovary and testis were sectioned to 6um in thickness, and stained with Haematoxylin and eosin (H **and** E) **Bernet *et al.* (1999**). They were examined and photographed by a built in Camera.

Water quality monitoring

Water temperature at 20cm depth (TC°) was recorded **daily** with a temperature meter at 9.00h, range **ranged** (31.9-25.3 C°).

Dissolved oxygen (DOmg.L^{-1}) was measured by instruction Manual, Hanna instruments (HI9146), USA (Dissolved oxygen and temperature meter) a weekly (range 6.3-4.5mg/L). Salinity (‰) was measured by Manual Handle ding consort (C 860) bvba, Belgium. a weekly (Range 22.1-14.5‰); pH was measured a weekly by Adwa instruments (A Dllo and A Dlll) pH meters Hungary a weekly (range 7.9-7.2)

Calculations

The following calculations were **used**:

Weight gain (%) =

(Final body weight – Initial body weight) ×100 / Initial body weight.

Feed efficiency (%) =

(Fish weight gain ×100) / Feed intake (dry matter).

Daily feed intake (percentage of body weight) =

Feed intake (dry matter)×100/[(initial fish wt. final fish wt.) x days fed /2].

Protein efficiency ratio (PER) =

Fish weight gain / Protein intake.

Condition factor (CF)

(Body weight ×100) / Total body length $(cm)^3$

Specific growth rate (SGR %) =

[(Ln Wf – Ln Wi) / T] ×100).

Where Wf and Wi refer to the mean final body weight and the mean initial weigth, and T is the feeding trial period in days.

Survival rate (%) =

(TNf / TNi) ×100

Where the **TNf** is total number of fish at finish and **TNi** is total number of fish at start.

Daily growth index, cited by **Kaushik *et al.* (2004),(DGI)=**

100 × (Final body weight$^{1/3}$ *– Initial body weight*$^{1/3}$*) / Time days)*

Mean weight (Geo metric mean),cited by **Lupatsch *et al.* (2001),=**

[(Initial body weight (g)) × (Final body weight (g))]$^{0.5}$

Total feed intake per fish =

Total feed intake (g) / Number of fish.

Protein intake =

[Feed intake (g)] × [Protein in the diet (%)].

Hepato-somatic index (HSI) =

100× (Liver weight (g) / Whole body weight (g)).

Gonado – somatic index (GSI) =

100 × (Weight of gonads(g) / Whole body weight(g)).

Statistical analysis

All data on fish growth performance, feed utilisation and muscle traits were statistically analysed by one-way analysis of variance (ANOVA), using **tests performed by Duncan (1955)** for individual comparison ($P< 0.05$) level of significance). Statistical analysis were carried out using the **IBM SPSS statistic (2011)** programme, version 19.

3. Results and Discussion:

The interpretation of data should be done with care because of some major problems encountered which led to experimental errors that could have masked the treatment effect. Fishery products, either in the form of low-value trash fish or rendered as fish meal, are presently the major sources of protein in the grow-out culture of most fish species and constitutes up to 70% by weight of their diet (**Tacon, 1995**). A dependable supply of cost-effective, non-marine alternative sources of protein must be provided for fish farming to be profitable **(Millamena, 2002)**.

The results presented in table (4) demonstrated effect trash fish on growth during 140 days (20 weeks). One reason for this duration, **Yigit *et al.* (2003) showed** that studies of protein and amino acid requirements of fish are usually conducted for an experimental period of 8-12 weeks. It is that statistical differences in the commonly measured growth criteria may not become apparent. Other responses such as feed intake and feed efficiency are not reliable criteria because of the difficult in collecting accurate data **(Lovell, 1989)**.

Specific growth rate, weight gain percentage, mean weight (geometric mean) and daily growth index were equation to examine growth performance of European sea bass fed the dry pellet or local trash fish with four different feeding schedule (Table 4).The final body weight and gain of sea bass fed a treatment (FS 5.4) was significantly increased (P< 0.05) than fish fed only local trash fish treatment (6, fed only trash fish). However, **there** was no significant differences (P > 0.05) among treatment 5, feeding schedule 4, and treat fish fed only dry pellet diet (DP)respectively.

Literature data on the growth of Mediterranean marine **finfish** species appear to be rather highly variable, probably due to differences in fish strains, water quality and temperature, oxygen availability, biomass density and biological value of dietary protein and non-protein energy substrates (**Company *et al.* 1999)**. In the present study, SGR (1.194-1.140% day) of European sea bass (Table 4) were better or within the range of previous study (1% by **Russell *et al.* 1996**; 0.7-0.8% by **Dias *et al.* 1998**, however 1,6-2% by **Perez *et al.*, 1997**).

In this connection, **Tubongbanua (1987)** study development of artificial feeds for sea bass (*Lates calcarifer*) who found that the mean biomass production of sea bass fed a trash fish diet was 71.7 kg/h for pasteurized trash fish diet, 70.5 kg/ha for sun-dried trash fish diet, 70.5 kg/ha for sun-dried trash fish, and 63.4 kg/ha for raw fish. **They also** showed that sea bass fed the pelleted fish meal had a production of 7.04 kg/ha while mashed fish meal gave only 56.4 kg/ha. These results appeared that the pasteurized trash fish had a better production compared to raw and sun-dried trash fish, this may be attributed to the deactivation of enzyme thiaminase and destruction of some harmful bacteria during heating, thus improving the feed quality of pasteurized trash fish over that of sun-dried and raw trash fish. This observation was also reported by **Lovell (1979)** for catfish fed with pasteurized, raw and sun-dried catfish waste. This may be due to some unknown growth factors in trash fish which could have been degraded during processing it into fish meal, the differences in enzyme thiaminase activity, or due to differences in the organoleptic attractiveness of the two products (**Tubongbanua**, **1987**).Though, **Fagbenro (1994)** found that tilapia fed dried fermented fish silage, can assimilate protein as amino acids and short peptides, so the protein breakdown during the treatment could have a positive effect on the digestibility and assimilation of this nutrient. In the present study considering the high acceptability of the trash fish diets, the use of trash fish in diets might act as a natural feed stimulant **(Plascencia-Jatomea *et al.*,, 2002)**. Though, they reported that small amounts of silage improved the growth efficiency of Atlantic salmon. This behavior was explained by the authors through the low levels of proteolytic enzymes within the gastro intestinal tract of the fry.

Results presented in table (4) showed that specific growth rate (1.168%/day) of fish had dry only dry pellet diet (Treat 1) control (1) decreased than treat 2,3,5 respectively. This is may be due to increased dry pellet intake 168.84 ± 7.48g as fed (Table 5) which **contained** some vegetable ingredients (Table, 2). A similar trend was reported by **Geay *et al.* (2010)**, **who** examined regulation of FADS2 expression and activity in European sea bass (*Dicentrarchus labrax*, L.) fed a vegetable diet. They observed a significant difference (P < 0.05) in fish final weight between dietary treatments with values of + 17.5% for fish fed fish diet by comparison fish fed 100% vegetable diet. This diminution of growth performance can be correlated to the significant decrease in n-3 HUFA content observed in flesh of fish fed vegetable diet (**Parpoura and Alexis 2001)**.Another reason could be linked to the low arachidonic acid content of the vegetable diet compared to fish diet. Indeed, an arachiedonic acid requirement has been reported for growth in different fish such as turbot (**Castell *et al.*, 1994**). Though, the supply of protein as vegetable meals could be another reason explaining the lower final weight of the European sea bass (**Geay *et al.*, 2010)**, since it has been previously shown that fish meal replacement by vegetable protein can reduce **growth** in this species **(Tibaldi *et al*. 2006)**.The lowest growth trend was obtained with treatment (6 fish fed only local trash fish) (Table 4) may be to increase chitin (scales) have an adverse effect on body weight as shown in studies reported by **Plascencia-Jatomea *et al* (2002)**.

Results presented in table (4) showed that final body weight, weight gain and specific growth rate were slightly decrease in sea bass (*D. labrax*) fed only dry pellet diet than fish fed schedule FS4(2 day dry pellet diet+ 1 day trash fish). This is may be due to increase carbohydrate intake (Fig.1) with fish fed only dry pellet diet(DP) than other treatment. There is a general consideration that carnivorous fish like European sea bass do not digest carbohydrates very efficiently **(Krogdahl *et al.* 2005)**. Moreover, dietary starch level may negatively affect carbohydrate digestibility and may also interact with the digestibility of other dietary nutrients **(Stone, 2003)**.

According to **Enes *et al*. (2006) who** did not observe any growth improvement in European sea bass juveniles fed diets including carbohydrates comparatively to fish fed a carbohydrate-free diet. Also, **Altan and Yildirmkorkut (2010)** noted that dietary starch level (10-30%) did not affect growth with sea bass (*D. labrax*).

The gain and the specific growth rate was significantly ($P < 0.05$) lower in fish fed only trash fish (T6) (Table 4), it could be due to the high level of lipid intake (94.46g) (Fig.1) which inhibiting the transdeamination of amino acid**,** this has also been reported by **Giri *et al*(2000).**

However, a lower of growth (gain and SGR) was noticed in fish fed on only pellet dry diets reported that it may cause decreasing the enzyme activity (Protease activity) with fish fed on plant protein **(Venkatesh *et al*., 1985)**. Though, a decrease growth in treat (1) may be due to increase carbohydrate/protein intake ratio 0.273 in treat (1) (Fig.1) which correlated inversely with apparent digestibility coefficient **(Fernandez *et al*, 1998)**.

Differences **in** weight gain and SGR between of the sea bass fed only trash fish for (Treat. 6) or fed only dry pellet diets (Treat 1) (Table 4) were significantly ($P < 0.05$) which may be affected by dietary moisture content. (Table 3). In this connection, effect of dietary moisture content on performance of fish varies depending on fish species **(Lee *et al*., 2000**) **who** reported that increase of moisture up to 30% of the diet reduced weight gain of Juvenile Atlantic salmon.

Though, **Ekanem 1996)** reported that estuarine catfish fry (*Chrysishthys nigrodigitatus*) grew better when fed a dry diet than moist diet, which probably results in improved water quality in the rearing pond. **Munsiri and Lovell (1993) showed** that the properties of the diet such as its composition of ingredients or its moisture content is affected on the gastric content of fish. Moreover**, Higgs *et al*. (1985) reported** that marine fish prefer moist feed to dry feed for its osmoregulation between body and medium, so that dietary moisture content may affect the performance of fish.

These facts reveal the increased weight gain of European sea bass (Table 4) **treated with** the feeding schedule 4(2 day dry pellet diet with 1 day trash fish) are mainly attributed to using a blend of dry pellet diet and trash fish which can be used to balance dietary essential amino acid content. In this connection, essential amino acid (EAA) deficiency is one of the factors limiting the utilization of economics protein **sources** as fish meal substitutes **(Glencross *et al*., 2007)**. Compared to fish meal, poultry by products meal (BM) and, meat and bone meal (MBM) are lower methionine and lysine contents and feather mea (FM) lower in methionine, lysine and histidine, (**Hertrampf and Piedad-Pascual, 2000)**. Also, blood meal **is** rich in lysine and can be used to balance dietary **lysine** content when poultry by product, meat and bone meal and feather meal are used, alone or in combination, as fish meal substitutes **(Milliamena, 2002; Guo *et al*., 2007)**.

Decrease weight gain (Table 4) of *Dicentrarchus labrax* fed only dry pellet diet (T1) may be attributed to incorporated meat and bone meal (MBM) in diet. **Robaina *et al*. (1995)** reported gilthead sea bream had low digestibility to the diets incorporated in MBM. **Millamena (2002)** suggested that high ash content in diets (content meat meals) may lower the digestibility of the diets that may have further caused the lowering in growth rates. However, **Xue and Cui (2001)** found that destroyed palatability has been demonstrated responsible to the reduced feed intake and growth of fish fed the diets in which high levels of dietary fish meal was replaced with economic plant or animal protein ingredients.

The **reduced** growth of *D.labrax* fed only dry pellet diet (Treat, 1) Table (4) than fish fed schedule 4 may be attributed to increase feed consume from fiber (11.929) for fish treat.2(Fig.1)**. Oberleas and Harland, (1977) showed** that a higher fiber, content in diet which can decrease transit time of intestinal contents (increase fecal nitrogen and lipid excretion as reflected by lowered protein and energy digestibility. Though, **Poston (1986)** observed for rainbow trout a growth depression with a level of cellulose incorporation above 8%. **Shiau and Liang (1994)** reported a better utilization of dietary protein when they tested agar supplementation at two dietary protein levels in hybrid tilapia. They suggested that this effect could be related to a slower feed passage time through the gastro intestinal tract, that might enhance overall nutrient absorption. However, **Dias *et al*. (1998)** found that the dietary of silica, cellulose or natural zeolite as bulk agents at 10 and 20% level had no adverse effect on sea bass (*D. labrax*) growth.

In the present study**,** fluctuation in water salinity (range 22.1-14.5per thousand) may **affect** on growth and feed consumption. **In their study**, **Partridge and Jenkins(2002)** found that, the European sea bass fingerlings grew about 20-30% more in sea water than those reared in fresh water. They show that the reason for this is thought to be due to the fact that fish in fresh water spend more energy than those in sea water for osmoregulation as **freshwater** species have to excrete higher amount of water from body to the hypo-osmotic environment. Though, different kind trash fish may effect on growth performance. **Hung and Mao (2010)** found that different kind trash fish (used three species of trash fish: A-anchovy, L-Lizard fish; C-cardinal fish) and those combinations (50% A+ 50%L; 50% A + 50% C; 50% L + 50% C). **These** different diets had significant differences on weight gain; specific growth rate, feed conversion ratio and survival of juvenile cobia (P < 0.05). Though, whole body proximate composition (crude protein, lipid and moisture) of cobia was affected as different fed trash fish. **Asturiano *et al.* (2001)** fed male European sea bass (*D. labrax*) on a wet diet (WD) consisting of trash fish 71% crude protein bogue (*Boops boops* L.). squid 79% crude protein (Loligo vulgaris) and fed two commercial **pelleted** diet (53% crude protein) enriched with polyunsaturated fatty acids (PUFA). They reported that the improved growth of the WD fed fish could be related to the higher dietary protein level of their food. Though, the same authors found that the WD diet consisted of two components bogue and squid, with the total percentages of saturated, monounsaturated and polyunsaturated fatty acids similar respectively in both components and the total percentage of PUFAs was highest in WD which also contained the largest percentage of total n-3 PUFAs as compared to two commercial pelleted diet. They added that since total lipid content was similar in three diets (wD and two commercial diets), it is possible that the PUFA lipid composition may be influencing reproductive performance.

A little variation in the survival rate in **the** treatments (Table 4) was observed. **Teng and Chua (1978)** noted that starvation occurs among fish populations where size hierarchy was established. The physical presence of large individuals inhibited the smaller fish from feeding satisfactorily and the dominance of feeding and spacy by a few larger fish in a restricted culture are can cause death of smaller individuals due to starvation. Further, it was also possible that the larger sea bass (*Lates calcarifer*) preyed on the smaller ones thus resulting in a decline in survival on some of the treatments (**Tubongbanua,1987**).

The proximate analysis of **musculature** of the sea bass (*Dicentrarchus labrax*) at different feeding schedules are shown in Table (5), insignificant effect on the moisture,protein and lipid content (P > 0.05) in both treatments (treat.6 or treat.1) (Table 5). With increase dietary moisture content in fresh fish (T.6) comparatively by sea bass (*D. labrax)* fed only dry pellet Table 3) diet (T.1) had no significant effect (P > 0.05) on proximate composition of dorsal **musculature** (Table 5).

A similar trend was found by **Lee *et al.* (2000)** who **examined the** effect of dietary moisture content on growth body composition and gastric evacuation of juvenile Korean rockfish *(Sebastes schlegeli)*. **Also**, **they showed** that dietary moisture content had no significant effect on protein and lipid content of muscle (P > 0.05).

Muscle body proximate composition of European sea bass *(D. labrax*) was presented in Table (5). Compared with body composition of sea bass (*D. **labarx***) at beginning trial, crude protein, and lipid concentration increased while ash and moisture concentration decreased at the end of experiment when fish fed only trash fish. On the contrary, **Hung and Mao (2010)** who noted that whole body proximate composition of juvenile cobia fed trash fish was differences at beginning trial than at the end of experiment. According to **Huy (2002) who used** trash fish that had low lipid content, caused lipid reducing in muscle of cobia. **Grigorakis *et al.* (2003)** show that muscle proximate composition of wild fish gilthead sea bream *(Sparus aurata)* were found to have lower lipid and higher water content than cultured fish.

With increase feed intake of lipid in *D. **labrax*** fed only trash fish (Table 5) had decreased concentrations of body fat (Table 5). **Alvarez *et al.* (1998)** found that European sea bass **revealed** no significant effect of dietary fat (8-18%) with respect to the total and neutral intramuscular fat content in the dorsal muscle was detected.

Crude protein content of muscle European sea bass (Table 5) was not affected by the various fed schedule treatments. This agrees with **the** finding where by feed compositions were observed to have relatively little effect on the whole-body protein of humpback groupers **(Shapawi *et al*., 2011).**

Feeding fish with local trash fish (Treat. 5) resulted in slightly higher body moisture and the lowest of body lipid content compared to European sea bass fed only the pelleted feeds (treat. 1), table (5). Moreover, these results corroborate previous reported data (**Shapawi *et al.* 2011)**. Concerning the effects of feeding consume (dry basis), protein and lipid content in muscle *D. labrax* was slightly **increased** with increase feeding consume for fish treat 5 (FS.4) comparatively by treat.1(DP) (Fig.1). No clear trend in moisture and ash content in sea bass **musculature** was observed at the end of the growth period (Table 5). This situation has already been demonstrated with sea bass by **Hidalgo *et al.* (1987)**, in which case, an increase in feeding rate from 0.74 to 1.45% bw day^{-1} caused a rise in the lipid content of the fingerlings at 15C°, whereas no significant effect of ration on the lipid content (1.0% to 2.6 bw day^{-1}) was detected at 20C°. **Mihelakakis *et al.* (2002) and Van Ham *et al.* (2003)** found that body lipid is reduced when the feeding rate is lowered. A similar trend was reported by, **Hillested and Johnsen (1994)** who noted the fat in the fillet was higher as protein content of the diets decreased to 35%, which corresponded to a Digestible crude protein/ Digestible energy ratio of 14.8. Under protein restriction, **Schwarz *et al.* (1985)** found that carp accumulated proportionally more dry matter, fat and energy. **Kaushik and Luquet, (1984)** who **fed** trout adlipitium with a non-protein energy source and restricted protein led to increasing levels of fat in the carcass.

Indices of condition, such as condition factor (K) and hepato-somatic index **(HSI)** are often used to assess the nutritional status of fish because they can be determined easily and may provide an indication of physiological condition (**Mihelakakis *et al.* 2002)**. In the current study at the end of the experiment, considering the result of condition factor, (Table 6) showed that *D. labrax* with FS.4 consumed more feed have slightly increase in CF than other treat. **Eroldogan *et al.* (2004),** show that an increase in feeding rate was reflect insignificantly increase in condition factor due to deeper body shape.

In the present study (Table 6) HSI with fish treat.5 (FS.4) had increased significantly ($P < 0.05$ respectively) with increase food supply (Fig.1).Similar observations on HSI have been reported in *Sparus aurata* (**Mihelakakis *et al.* 2002)** who found that **HSI** were significantly higher with feeding rate 2% / day, comparatively with feeding rate up to 0.57 % / day.Hepato-somatic index **(HSI)** in fish usually suggests problems in nutrition because the relative size of the liver is correlated with nutritional state of the fish. A similar decrease in **HSI** was observed in striped bass when feeding rate was reduced from 1.0 to 0.5 BW.d (**Hung *et al.*, 1993)**.On the other hand, hepato-somatic index in treat.1 (fed only dry pellet diet) was significantly ($P < 0.05$)**increased** than treat (6) fed only trash fish (Table 7)**. This** may be due to decrease fishmeal as protein source in dry pellet diet and substitute by plant protein source,soybean and corn gluten meal, (Table 2). **Dias, (1999) showed** that hepatic fat deposition indeed is high, there is evidence that replacement of fish meal by plant protein source such as corn gluten meal or soy protein concentrates affects hepatic lipogenic enzyme activities variably in sea bass, while the activity of malic enzyme decrease, that of fatty acid synthetase increased significantly with high level of corn gluten meal in the diet. **Shapawi *et al.* (2011)** stated that **HSI** is related to the nutritional state of fish and may directly related to energy requirements for growth **and found** that poor growth of grouper (*Cromileptes altivelis*) as a result of lower protein content and palatability of the feed probably had contributed to a higher value of **HSI**. However, they found that feeding fish with trash fish resulted in lower **HSI** compared to fish fed the pelleted feeds.Inthis trend, **Hernandez *et al.* (2007)** suggested that diets containing high amounts of carbohydrates should result in higher **HSI** index. On the other hand, feeding rate and feed consume not only feed conversion efficiency but also *condition factor* and *hepato-somatic* index, which are used to assess the nutritional status of fish and are good predictors of physiological condition of aquatic animals **(Ng *et al.*, 2000; Mihelakakis *et al.*, 2002).**Though, a similar trend with hepato-somatic index in the present study (Table 6). **Hung *et al.* (1993)** in striped bass and **Mihelakakis *et al.* (2002)** in gilthead sea bream, observed similar differences in the condition in dices of fish fed suboptimal and adequate rations were reported when feeding rate was reduced from 2.0% to 0.5% body weight/day.

At the end of the 140 days of the growth trial, all groups of *D. labrax* **showed** mean individual gonad somatic index ranging from 0.281 to 0.27% for ovary, and 0.0597to 0.0566% for testes, (Table 6) with no significantly differences among groups. Though, results presented in table (6) showed that gonado-somatic index (ovary and testes) for *D.* ***labrax*** fed dry pellet diet (treat.1) or fed only local trash fish (treat.6) alone was decreased insignifican**tly** ($P > 0.05$) than fish fed combination of dry pellet diet and local trash fish (FS.4). A similar tendency was also observed by **Teruel *et al.* (2001) who** reported that a higher amount of essential nutrients in the artificial diets such as protein, lipid and the highly unsaturated fatty acids, for example, 20:4 n-6, 20: 5-n-3, 22: 6n-3 in *Holiotis asinina* fed artificial diet alone and a combination of natural diet and artificial diet influenced the increased reproductive performance (gonado- somatic index).In this connection **Kaushik *et al.* (2004)** found *Dicentrarchus labrax* fish meal replacement by plant ingredients (fish meal was decreased gradually from 100% to about 2% and replaced by plant protein sources were formulated have a slight increased in gonado-somatic index (GSI) 0.52, 0.72, 1.41. When final body weight 330.8; 333.2, 317.2g; with fish med was decreased gradually u to 25% (Fish meal 52, 40, 25) replaced by plant protein sources. **Cerdá *et al.* (1995)** with female bass (*D. labrax*) showed that fish fed with krill before or during the spawning season produced egg and larvae with a better quality than fish fed trash fish. This positive effect has been attributed to the presence of phosphatidy choline and astaxanthin from polar and nonpolar lipid fractions of raw krill, respectively **(Watanabe *et al.*, 1991 a,b**).

Previous studies involving examination of female (*Dicentrarchus labrax*) reproductive performance, the wet diet proved to be the most beneficial **(Navas *et al.*, 1998)** contrary to the results obtained by **Asturiano *et al.* (2001)** for sea bass (*D. labrax*) **males**. Since steroidogenesis and eicosanoids are essential for testicular function, it is possible that contrary to what has been reported in females, a reduction in these n-3 dietary PUFAs and an increase in AA (arachidonic acid) resulting in an altered n-3: n-6 PUFA ratio may improve male European sea bass reproductive performance. Though, the same authors found that European sea bass fed commercial PUFA-enriched diets exhibited enhanced reproductive performance as compared to fish fed a wet diet. Studies have shown that male European sea bass fed diets with varying levels of n-3 and n-6 PUFAs exhibit depressed testicular steroidogenesis which may result in a delay in timing and extent of spermiation **(Cerdá *et al*., 1997)**. Moreover, if the in vitro studies **(Wade *et al*., 1994)** showing the regulatory effects of AA, arachidonic acid, EPA, eicosapentaenoic acid, and DHA, docosahexaenoic acid, on testicular function are taken into account. The same authors shown that AA stimulates testosterone production, whereas n-3 PUFAs, particularly EPA, may function as inhibitory regulators. Recent in vitro studies in European sea bass testis demonstrated that AA-stimulated PG (prostaglandins) series E (PGE2) production was enhanced in the presence of gonadotropin, while EPA and DHA were ineffective **(Asturiano *et al*., 2000)**. It has been proposed that the inhibitory actions EPA and DHA may in part be due to inhibition of PGE2 formation form AA **(Wade *et al*., 1994)**.In addition, high cellular levels of EDA and DHA are known to displace AA and compete for cyclooxygenase enzymes which convert these PUFAs to eicosanoids which are less effective than PGE2 in reproductive events **(Murdoch *et al*. 1993)**. In this regard, results by **Asturiano *et al*. (2001)** show that sea bass (*D. labrax*) fed diets lower in EPA and DHA composition and n-3:n-6 ratios had enhanced reproductive performance.**Chaitanawisuti *et al*. (2011)** attempted to condition broodstock spotted babylon (*Babylonia areolata*) using formulated diets than a local trash fish. They found that the levels of protein content of the formulated diet did not differ significantly among the local trash fish, but lipid content and total unsaturated fatty acids of the formulated diet was significantly higher than those of the local trash fish. On the other hand, they showed that the proximate composition of egg capsules (Protein and lipid) revealed that there were no significant differences fed both formulated diet and local trash fish but significant differences in fatty acid composition (higher levels of EPA, DHA and ARA) than those of broodstock fed the trash fish.

One of the most important factors necessary in the successful culturing of a fish species is obtaining a basic understanding of its key biological processes. The most important of these biological processes in the fish's reproductive cycle and formation of gametes. Small,round and transparent oocytes with a central nucleus were observed in histological sections of ovaries (plate 1: a, b,c, d, e, f) and nucleoli were found in few oocytes. Also,no lipid droplets were found. These cells had basophilic cytoplasm and an acidophilic nucleus (plate 1. a, b, c,d, e, f).Though, in the present study, testes, based on microscopic observation were distinguished for male *Dicentrarchus labrox* Plate 2 (a, b, c, d, e, f) seprmatogonia and primary spermatocytes were the dominant cells of this stage (plate 2).Though, spermatogonia had a light cytoplasm and a large nucleus. Some secondary spermatocytes having basophilic cytoplasm were also observed (plate 2).The results present in Table 6 and plate 1,2,show that fish fed mixed from **formulated** diet and local trash fish especially feeding schedule 4 have improving reproductive performance (gonado somatic index) and development tissue of gonads (ovary and testes) may be to different unsaturated acids content in both formulated diet and local trash fish. **Chaitanawisuti, *et al.* (2011)** found that the formulated diet contained significantly higher levels of ARA (arachidonic acid, 20:Ln-6), EPA (eicosapentaaenic acid, 20:5n-3) and DHA (docosahex aenoic acid, 22:6n-3) than those of fish fed the local trash fish. The same authors, showed the compositions of egg capsules produced significantly more ARA, EPA and DHA compared to broodstock fed the local trash fish,however, the ARA /EPA and DHA/EPA ratios in egg capsules were significantly higher in the trash fish – fed group- compared to those fed the formulated diet. **Lupatsch *et al*,(2001) reported** that good knowledge on nutrient requirements **is needed** for best growth performance. Though, feed ration effects a wide range of biochemical parameter, in fish which in **turn** help to formulate its physiological response to environmental stimuli **(Chatzifotis *et al*,2011).** The same authors, showed that a case in point is the development and maturation of gonads which is achieved by the transfer of nutrients from the diet /or somatic tissue under the influence of daylight and water temperature. On the other hand, with sea bass *(Dicentrarchus labrax)*,**Papadaki *et al* (2005)** observed precocious males and females reach puberty after the first and second year of life (300- 400g), respectively, diverting a part of surplus domatic energy to reproduction instead of somatic growth. **Cerda *et al*(1994)** reported that reduced feeding can delay the timing of spawning in sea bass and decrease fecundity, but with the concomitant reduction of somatic growth,however, gonadal maturation is accompanied by the transfer of lipids from energy increase of plasma lipids **(Fernandez *et al,*1989)**. However,effects of starvation and re-feeding on reproductive indices long term starvation (one month feeding –three month starvation) and starvation re-feeding (two month starvation –two month re-feeding) on gonad maturation for *Dicentrarchus labrax* (12 months old, weighing 289 ± 34 gwere examined by **Chatzifotis *et al* (2011), who** showed that the feeding regime had a profound effect on gonad maturation of sea bass,male and female fish in the long term starvation feeding regime show significantly lower gonado somatic index values than those on the starvation / re-feeding, feeding regime.

Table (1): Nutrient composition (%) in dry matter of feedstuffs

Test ingredients	Dry matter	Crude protein	Crude lipid	Crude fiber	Nitrogen free* extract	Ash
Fish meal	89.9	72.86	10.23	3.12	-	13.79
Meat and bone meal	92.3	50.70	7.91	3.90	16.58	20.91
Soybean meal	89.7	44.04	4.24	7.92	35.23	8.58
Corn gluten meal	88.2	56.24	5.90	4.76	26.08	7.03
Wheat flour	88.0	18.18	3.41	8.52	52.16	17.73

Table (2): Test diet formulations (pelleted diet)

Ingredient	Amount (g/100g diet as fed)
Fish meal	36.5
Meat and bone meal	20.7
Soybean meal	12.3
Corn gluten meal	8.0
Wheat flour	14.5
Fish oil	5.0
Vitamin*	1.5
Mineral**	1.5

* Vitamin premix (local) supplied the diet (g/kg) the following: Vit.A,9000000. I.U.;
D_3, 1500000 IU; E, 24g; C, 6g; K3, 1-2 g; B1, 0.9g; B2, 1.5; B6, 1.2g; Folic acid, 0.12g Niacin, 6g; Pantothenic acid, 2.76g. (Carrier, cellulose, up to 1000g).
** Mineral premix (local) consisted of (mg/kg premix) the following: 4000mg KCL (52%); 1030 mg $ZnSO_4.7H_2O$; 33mgKI; 1.35mg $Na_2Se_2O_3$; 1319mg $MnSO_4.H_2O$; 50mg copper sulphate (25% Cu); 5mg cobalt sulphate; 4300mg sodium sulphate (32.37% Na). (Carrier up to 1000gm).

Table (3): Proximate compositions of pelleted feeds and local trash fish

Parameters	Pelleted feed	Trash fish
Proximate composition (g/100g diet)		
Dry matter	90.77	25.46
Crude protein	49.29	61.63
Crude lipid	12.33	16.73
Crude fiber	7.78	-
Ash	17.14	19.40
Nitrogen-free extract	13.46	2.24
Gross energy (MJ/Kg^{-1})*	19.06	21.78
Protein energy ratio (mg protein/KJ)	25.86	28.30

Gross energy calculated using: 23.9 KJg^{-1} protein; 39.8 KJg^{-1} lipid; 17.6 KJg^{-1} carbohydrates (cited by **Martinez-Liorens *et al*., 2007**).

D. labrax is already known to exhibit sexual growth dimorphism at commercial size (300 – 400 g). The females are larger than males at this stage of development with a relative advantage estimated at 20 - 40% **(Carrillo *et. al*., 1995; Chatain *et. al*., 1997). Saillant *et. al*., (2003)** reported that high densities and size grading applied to intensive sea bass are not responsible for the sexual dimorphism in farmed populations. However, the feeding rates and growth conditions of fish may account for a part of the sex ratio variation. Results obtained from this study show that there are histological features in the gonads of the males and females *D. labrax.* Sea bass culture, however, is dependent on the supply of trash fish because of the unfavourable response of the fish to artificial pelleted feed. **Suzuki, *et al*. (2000)** found that, the thickness of the Zona radiata is probably an adaptation to protect the developing egg from injury and from abrasion. The rhythm of deposition of yolk inclusions in the oocyte of fish differs from species to species. In *Oreochromis mossambics*, lipid vesicles and yolk granules appear in the oocyte at the same time **(Dadzie, 1974). Lasiak (1983)**, observed progressive thickening of the zone radiata with maturation in the mullet, *Liza richardsoni*, in connection with substratum spawning. In other teleosts, lipid vesicles are the first type of yolk inclusion to appear in vitellogenic oocytes, their appearance marking the **onset** of vitellogenesis **(Schackley & King 1977, Wiegand, 1982, Dadzie, *et. al*., 2000). Dadzie *et al*., (2000)** did not observe cortical alveoli either, in the occytes of ***Pampus*** *argenteus*. The authors, however, reported the presence of lipid-containing vacuoles in pre-vitellogenic oocytes. These vacuoles disappeared from the cells prior to the onset of vitellogenesis. It is possible, consequently, that in the present study, cortical alveoli were also transient as a distinctive phase and, therefore, not seen. **Abou-Seedo *et. al*. (2003),** through the analysis of the gonadosomatic indices of the fish, adduced evidence to the effect that although mature individuals occur in December, their numbers are too low to take into consideration, in terms of the management of the *Acanthopagrus latus* species in Kuwait waters, and that the greater proportion of individuals being to spawn in January. **Lasiak (1983),** observed progressive thickening of the zona radiata with maturation in the mullet, *Liza richardsoni*, in connection with substratum spawning.

Table (4): Growth performance of *Dicentrarchus labrax* fed the dry pellet (DP) or local trash fish (TF) with different feeding schedules (FS). Values are means ± standard error of two replicate of net pen culture.

Diets and Feeding schedule Growth performance	Dry Pellet diet	Feeding schedule 1	Feeding schedule 2	Feeding schedule 3	Feeding schedule 4	Local trash fish
Initial body weight (1BW) (g)	22.56±1.56	21.77±2.2	21.62±1.95	22.73±1.13	23.01±1.84	21.80±2.14
Final body weight (FBW) (g)	115.75±2.74[ab]	115.90±3.07[a]	113.71±2.87[b]	112.13±3.11[bc]	121.75±3.25[a]	109.22±3.37[c]
Gain (g)	93.19±2.38[ab]	94.13±2.87[a]	92.09±3.01[b]	89.40±2.62[bc]	98.74±3.11[a]	87.42±3.25[c]
Weight gain (%)	413.08±15.8[b]	432.38±18.3[a]	425.95±16.8[a]	393.31±14.7[c]	429.12±18.9[a]	401.01±19.3
Specific growth rate (%/day)	1.168±0.07	1.194±0.05	1.186±0.06	1.140±0.08	1.190±0.05	1.151±0.07
Geometric mean (mean weight)	51.10±3.64[a]	50.23±3.41[ab]	49.58±2.29[b]	50.48±3.12[a]	52.93±.4.31[a]	48.80±3.56[b]
Daily growth index (%)	1.46±0.08	1.49±0.07	1.47±0.05	1.42±0.007	1.51±0.05	1.42±0.08
Survival rate (%)	90.0±2.5	82.5±5.0	85.0±2.5	90.0±7.5	90.0±2.5	82.5±5.0

Mean With different superscript letters within rows are significantly differently ($P < 0.05$)

Table (5): **Proximate analysis (%) of dorsal muscle in European sea bass on wet tissue fed the dry pellet (DP) or local trash fish (TF) with different feeding schedule (FS) (n=2, each n consist of measurement of 5 fish).**

Proximate analysis (%) Diets and feeding schedule	Moisture	Crude protein	Crude fat	Ash	Gross energy (KJg^{-1})
Initial	76.27±2.35	18.34±0.94	2.16±0.18	3.21±0.22	524.3±23.1
Dry pellet diet (DP)	75.01±2.24	19.07±1.02	3.39[b]±0.21	2.63±0.20	590.7±19.7
Feeding schedule.1 (FS_1)	74.36±1.96	19.11±0.87	3.85[a]±0.24	2.68±0.17	610.0±18.3
Feeding schedules 2 (FS_2)	74.39±2.07	19.22±0.95	3.77[a]±0.19	2.62±0.21	60.4±25.01
Feeding schedule 3 (FS_3)	74.95±2.15	19.19±1.10	3.45[ab]±0.22	2.40±0.19	596.0±2.23
Feeding schedule 4 (FS_4)	74.40±1.88	19.25±0.98	3.70[a]±0.15	2.66±0.20	607.3±19.8
Local trash fish (TF)	75.58=2.17	19.10±1.21	3.18[b]±0.20	2.14±0.18	583.1±2.15

Values are mean ± standard error values in the same row with different superscript letters are significantly different ($P < 0.05$); DM = dry matter

Table (6): Somatic parameters of European sea bass fed the dry pellet (DP) or local trash fish (TF) with different feeding schedule (FS). Values are mean ± standard error of two replicates values in row with the same letter are not significant ($P \leq 0.05$)

Somatic parameter Diets and Feeding schedule	Condition factor (K)	Hepato-somatic index %	Gonado-somatic index	
			Ovary	Testes
Dry pellet diet (DP)	1.21±0.08	1.38±0.05[a]	0.279±0.0012 (n = 20)	0.0584±0.0008 (n = 10)
Feeding schedule 1 (FS_1)	1.23±0.10	1.33±0.07[bc]	0.276±0.0064 (n = 24)	0.0585±0.0009 (n = 6)
Feeding schedule 2 (FS_2)	1.22±0.11	1.35±0.04[ab]	0.278±0.0004 (n = 21)	0.0579±0.0014 (n = 9)
Feeding schedule 3 (FS_3)	1.19±0.07	1.34±0.07[b]	0.271±0.002 (n = 23)	0.0574±0.0014 (n = 7)
Feeding schedule 4 (FS_4)	1.25±0.08	1.41±0.08[a]	0.281±0.0011 (n = 19)	0.0597±0.0013 (n = 11)
Local trash fish (TF)	1.20±0.09	1.30±0.06[c]	0.275±0.003 (n=22)	0.0566±0.0016 (n = 8

Data are presented as mean ± S.E., n = 30 fish; N= Number of fish

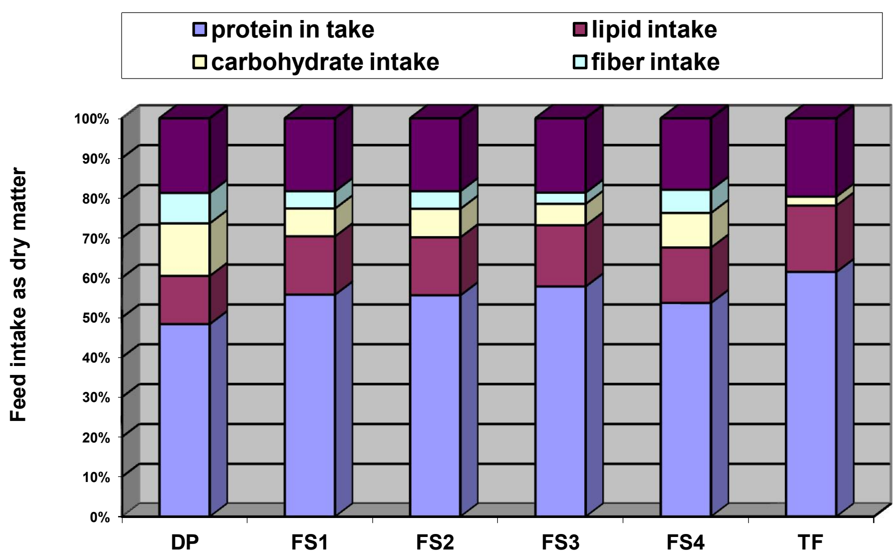

Fig. (1) Feed intake as dry matter for *Dicentrarchus labrax* as different feeding schedules

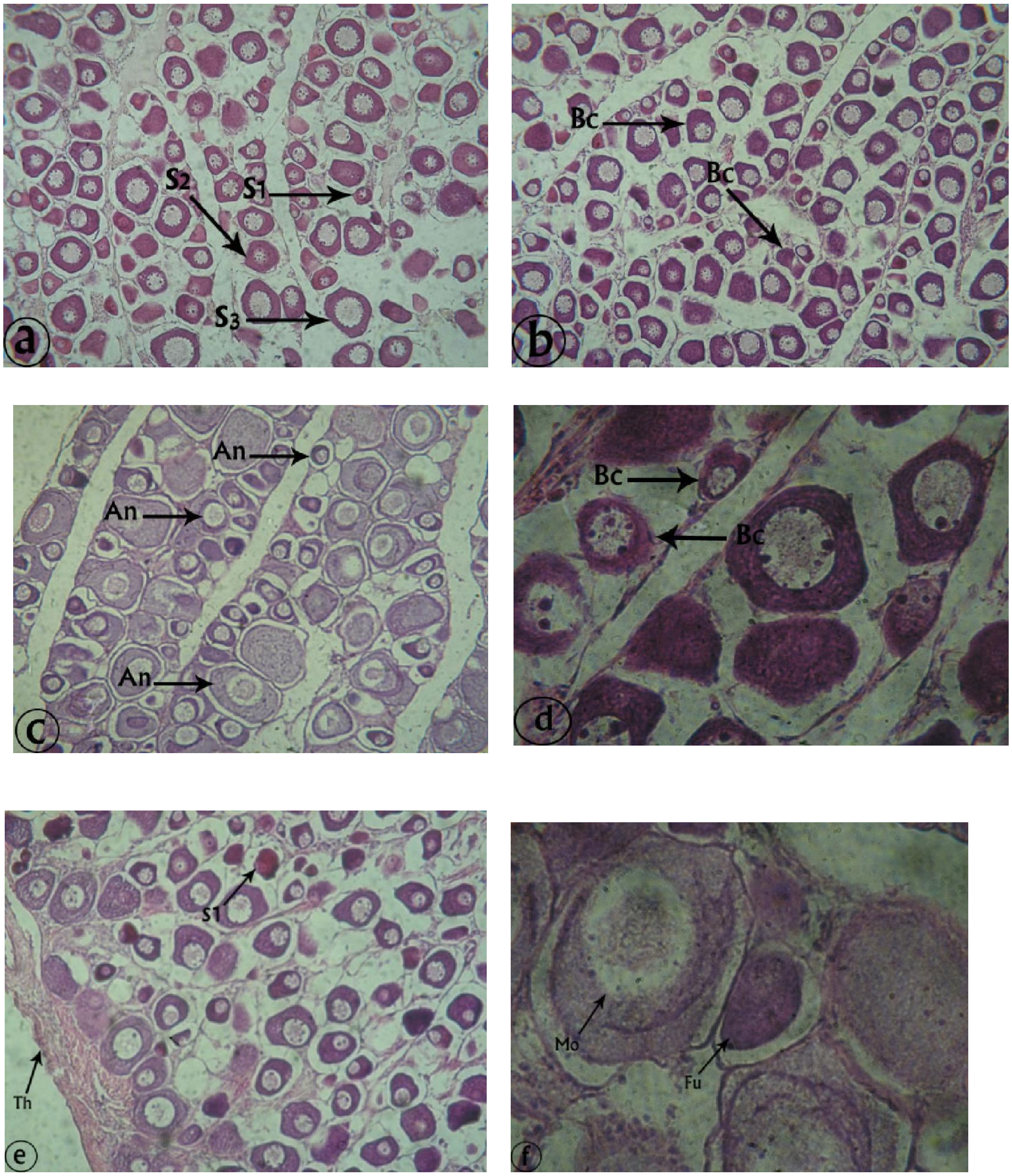

Plate (1).
Sections of Dicentrarchus labrax ovaries of fed different feeding schedule:
DP. a: Primary stage (S1), Secondary stage (S2) and Third stage (S3)
FS1.b: Basophilic cytoplasm(Bc) x100
FS2. c: Acidophilic nucleus (An) x100
FS3. d: Basophilic cytoplasm(Bc) x200
FS4. e: Theca layer (Th) and stage (S1) x100
TF.F: Mature ova (Mo) and fused two ova (Fu) x400

Small, round and transparent oocytes whith a central nucleus were observed in histologica sections of ovaries (plate-1) Nucleoli were found in few oocytes, No lipid were found, these cells had oasophilic cytoplasm and an acidophilic nucleus (plate -1,a,b,c,d,e,f).

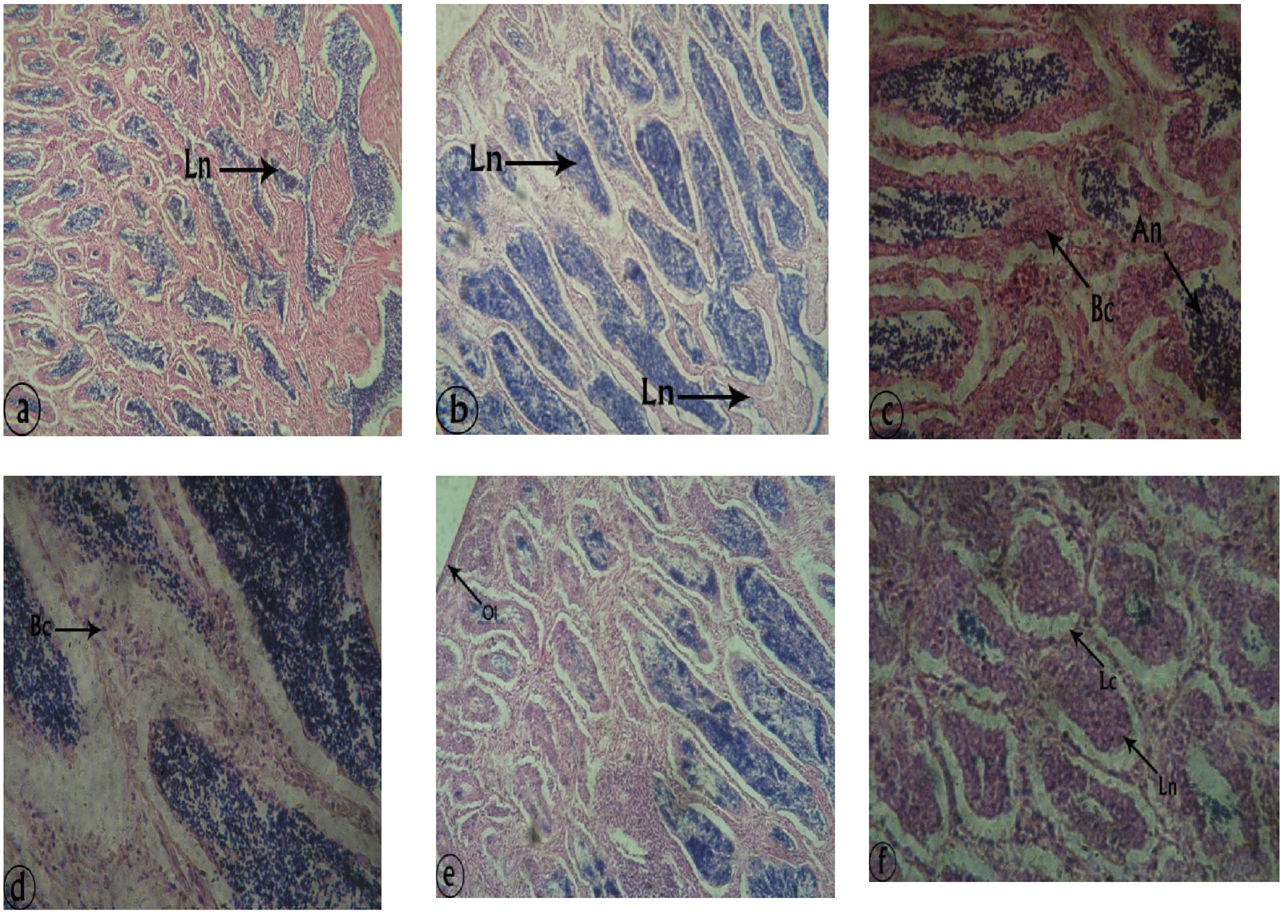

Plate (2).
Sections of *Dicentrarchus labrax* testes of fed different feeding schedule:
Dp. a: Large nucleus (Ln) x100
FS1. b: Large nucleus (Ln) x200
FS2. c: Basophilic cytoplasm (Bc) x200
FS3. d: Basophilic cytoplasm (Bc) x400
FS4. e: outer layer of testes (Ol) x200
TF. f: large nucleus (Ln) x200
Spermatogonia and primary spermatocytes were the dominant cells of this stage.
Thoughts spermatogonia had a light cytoplasm and a large nucleus,
some secondary spermatocytes having basophilic cytoplasm were also observed

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