Feeding European Sea Bass (*Dicentrarchus Labrax*) With Trash Fish 2- Nutritive Utilization and Histological Digestive Tract.

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Abstract: One of the major problems facing aquaculture is the raising prices of commercial feeds for European sea bass (*Dicentrarchus labrax*) and environmental risk with using inadequate supply of ingredient diets. An 20- week feeding experiment was conducted on *D.labrax* (initial body weight, BW, 22.25±0.59g) to determine higher benefit of dietary content from local trash fish (feeding rate 8.6% of body weight) or dietary content from dry pellet (feeding rate 2.2% of BW.) individually and or with overlap in four feeding schedules (Fs): 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. Fish (in experimental diets) were maintained outdoor (40 fish / net pen culture) under natural condition of temperature and photoperiod with duplicate per treatment. The performance of feeding schedule compared with a dry pellet diet or local trash fish fed European sea bass was examined. Feed conversion ratio, feed conversion efficiency and protein efficiency ratio were better with fish feeding schedule 4, and fish was feed on only dry pellet diet comparison with feeding schedule (1.2.3). Protein, lipid and energy retention of *D. labrax* had significantly higher (*P*< 0.05) compared to fish fed only local trash fish. Histological observation in intestine of *D. labrax* reaction to fed dry pellet diet or trash fish with four feeding schedule. The degenerative changes included fused microvillii, the outer membrane of microvilli are broken cell swelling and hemorrhage in the submucosa regain with increased feed intake from dry pellet diet comparison to fish feed consume local trash.

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1. Introduction

European sea bass (*Dicentrarchus labrax*) is one of the most important aquaculture species in the Mediterranean countries **(Altan and Yildirimkorkut 2010**), and cultivated in the Mediterranean region **as** a euryhaline marine teleost species **(Dalla-Via *et al*., 1998; Varsamos *et al*., 2001). Also, it is** one of the most economically important fish species farmed in temperate areas **(Eroldogan *et al*., 2004).**

The use of trash as fish feed is being regulated in some countries. Formulated feeds are expensive as most of the ingredients are imported and prices are rising continually. It is necessary to seek cost-effective replacement to supply dietary protein from locally produced inexpensive materials in order to alleviate high feed costs. Therefore, feed quality and feeding strategy are great importance in fish nutrition science **(Houlihan *et al*., 2001).**One category of trash fish are those not used for direct human consumption, which may be either landed and discarded at the sea itself **(Chandrapal, 2005).** In Vietnam, the pellet feeds has been developed and used in cobia (*Rachycentron canadum*) culture, but due to difficulties in feed supplying and high prices so the farmers still tend to use trash fish in cobia farming because of stable supplies and low prices **(Hung and Mao, 2010).** Also, the same authors showed that due to difficulties in feed supplying and high prices so the farmers still tend to use trash fish as the main source feed for cobia grow-out (*Rachycentron canadum*) farming. **Bunlipatanon *et al*. (2014)** evaluated the efficacies of the use of commercial pellet feed in comparison to trash fish in cage aquaculture of Asian sea bass (*Lates calcarifer*) and tiger grouper (*Epinephelus fuscoguttatus*). They showed that the overall growth performance and fish survival rates between the two feed types were similar, for both species. **Yigit *et al* (2003) evaluated** three protein sources of trash fish whiting (*Merlangius merlangius*); goby (*Gobius sp.*) and anchovy (*Engraulis encrasicolus*) to see which best meets the nutritional requirements of young Black sea turbot. In shrimp farming, **Chandrapal (2005)** found that feed accounting for 50 to 70 percent of the total variable cost of production and that some the list of key ingredients used in the manufacture of shrimp feed are fish meal and trash fish.Two distinct feeding regimes are used for broodstock sea bass *(Dicentrarchus labrax)* maintenance, **Buke (2002)** fed fish with pellets at a rate of 0.5% of biomass/day, these pellets contains: Protein > 46%, lipid > 12%; if available, fresh feed (fish-by-catch) is given twice a week at rate of 1.0% biomass/day.

Using a combination of statistical and histological methods by **Segner and Juario (1986)**, they established that the optimal time for a feed change of *Chanos chanos* larvae is a few days prior to metamorphosis. The fish digestive tract shows a marked diversity of both morphology and function **(Kozaric *et al*., 2007)**. This is in correlation with the different feeding habits as well as with the body shape **(Buddingtion *et al*., 1997).** The structure of the digestive tract of teleosts varies with different factors **(Giffard-Mena *et al*., 2006).** Its function include digestion, nutriment absorption, hormone secretion, immune protection and water and salt transfers for hydro mineral homeostasis. It regulates energy and material exchanges between the environment and the internal medium. Its structure is also variable according to the nature of the diet. Rather short in carnivorous species (20% of body length) in herbivorous fish **(Buddingtion and Kuzmina 2000).** It comprises distinct portions: the mouth cavity, the esophagus, the stomach, the anterior and posterior intestine and the rectum, each one playing a role in ion and water regulation **(Ando *et al*., 2003).**Identification of digestive tract structure is essential for understanding the related histophysiological mechanisms and nutritional functions **(Khojasteh, 2012).** The same author, show that the anatomical and histological characteristics of fish intestine are expected to be helpful for understanding the related functional mechanisms and feeding habits, which can further be helpful for diagnosing some intestinal diseases and formulating suitable feeds. **Raškovic *et al*. (2011)** described the important of using histological methods to assess the effects of feed on the liver and intestine of fish. Histological analysis of the digestive system is considered a good indicator of the nutritional status of fish **(Caballero *et al*., 2003).** The intestine and liver are the most important organs in digestion and absorption of nutrients from food, and therefore monitoring of these organs is considered necessary **(Roberts, 1989).**Though, **Salamat *et al.* (2011)** reported that the histological study of fish gut is becoming more precious as the interest in fish culture increases and therefore, more information is necessitated with regard to feed and nutrition. The same authors, found that gut-associated lymphoid tissue (GALT) existed in all over the intestine (*Acanthopagrus Latus*), as single cells or small aggregations in both the epithelium and lamina properia. Though, lymphocytes, plasma cells, granulocytes and macrophages were recognized in the intestinal mucosa, lamina properia and submucosa of *Acanthopagrus latus*.

The **present work was designed to study the** existence of **harmony** and balance between reducing the costs of feeding sea bass (*Dicentrarchus labrax*) **for decreasing** the environmental risks, using two types of diets, dry pellet diet or local trash fish (as wet diet) with four feeding **schedules**.

2. Materials and Methods

Culture system, fish, diets and experimental design

The experiment was carried out in twelve (12) net pen culture (diameter 4.0m, depth water 1.2m.) inside commercial farm (earthen pond) at **Port Said** governorate, Egypt. Juvenile European sea bass (*Dicentranchus labrax*) were obtained from a local commercial fish farm (at **Port Said** governorate, Egypt. 480 homogeneous fish with **a** range initial body weight 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 was hand fed in plastic box. The feeding trials were conducted for 140 days (from June to October,2012).

The experimental 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.

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 of the local trash fish and formulate diet are shown in Table (1). All ingredient were grinding, mixture and processed into a California pellet Meal machine (CPM) as dry pelleted diet.

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 muscle composition analysis, weighed liver, and gonad (ovary, testis) for determine somatic-index and examine histological for gonad intestine.

Proximate composition and water analysis

Samples of feed ingredients diets and fish **musculature** 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. Nitrogen free extract (NFE) was computed by taking the sum values for crude protein, lipid, ash, fiber and moisture, and subtracting this from 100.

Specimens from intestine and stomach of *Dicentrarchus labrax* were fixed in 10% neutral-buffered formalin, dehydrated, embedded in paraffin wax and sectioned at 4-6 µm then stained with haematoxylin and eosin according to the method described by **Bernet *et al*. (1999).**

Water temperature at 20cm depth (TC°) was recorded daily with a temperature meter at 9.00h, range water temperature meter (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)

Data Calculations and Statistical analysis

The following calculations were made:

Weight gain =*(Final body weight – 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.*

Total feed intake per fish = *Total feed intake (g) / Number of fish.*

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

Feed conversion ratio (FCR) = *[Total dry feed intake (g) / Total wet weight gain (g)]*

Protein retained Protein deposition in muscle or protien productive value in muscle: (**Estefanell *et al*., 2011**) = *(Protein deposition in final muscle fish – Protein deposition in intial muscle) ×100/ Protein intake*

All data on fish growth performance, feed utilisation and muscle traits were statistically analysed by one-way analysis of variance (ANOVA), using **Duncan test (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 discrepancies between the results of our recent study and findings of others could be explained by size and/or age differences of fish and experimental conditions such as stocking rate, feeding regimes or water quality **(Webster *et al*., 2002; Fiogbe and Kestemont. 2003).**

In this study **it was revealed that** European sea bass (*D. labrax*), the feed consume was increased in fish fed schedule 4 (2 days dry pellet + 1 day fresh trash fish), than fish fed only dry pellet diet, (Table 2) it's may be due to palatability which related inverse with increased formulated with cellulose, (Table 1). According, **Bromley and Adkins (1984)**, trout could regulate their feed intake to make up for low energy density until 30% cellulose.

Feeding was also reduced in channel catfish, when given a diet with higher fiber levels **(Page and Andrews, 1973).**

Lupatsch *et al*. (2001) reported that feed intake per g BW $(kg)^{-0.70}$ decreased with high dietary digestible energy content. The same authors **showed** that gilthead sea bream was able, within limits, to compensate for a low energy feed by enhancing intake as long as the physical capacity of the digestive tract permitted and the feed intake indirectly controlled the protein consumption.A similar process was observed in trout, where caloric intake was regulated by feed, but protein intake was not compensated by feed intake **(Boujard and Médale, 1994)**. **Ellis and Reigh (1991)** also suggested in their study with red drum, that low protein intake in combination with high dietary gross energy: crude protein ratios was growth limiting.

In this connection, **Kentouri *et al*., (1995); Paspatis and Boujard (1996) reported** that digestible energy content is thought to be one of the major criteria controlling feed intake in fish along with other factors including fish size, temperature or palatability. Though, the lower feed intake of diets containing protein sources other than fish meal might be attributed to essential amino acid deficiencies, as shown for arginine **(Kim *et al*., 1992a)** Lucien **(Choo *et al*., 1991)** and methionine **(Kim *et al*., 1992b).** Where, normal intake values are reached only when the amino acid concentration in the diet meets the requirements of the fish **(De La Higuera, 2001).** On the contrary, when the European eel, *Anguilla anguilla*, was fed on diets containing sunflower protein supplemented with needed amino acids, feed intake and utilization reached values similar to those for fish fed on a fishmeal diet **(Garcia-Gallego *et al*., 1998). Sanchez-Muros *et al.* (2003)** show that inefficiency of essential amino acid supplementation might be attributed to an imbalance of postprandial amino acid availability due to the early absorption of dietary free-supplemented amino acids. In this sense, with the European sea bass **(Thebault, 1985)** and gilthead sea bream **(Sierra, 1995).**

A decrease daily feed consume of trash fish with Feed schedule(4): 2 day dry pellet diet with 1 day trash fish) (Table,2), than other treatments, may be the cause in **improving** feed efficiency and growth in this treat.

Thia-Eng and Seng-Keh (1978) found that the daily feed intake of young estuary grouper (initial size ranging from 16.2 to 16.9 cm in total length) was closely related to the amount feed remaining in the stomach and 95% of feed was digested in 36 h, when chopped trash fish was fed. As well as, **Grove *et al.* (1978)** showed that rainbow trout had more than 50% appetite return when more than 50% stomach content was emptied. **Lee *et al.* (2000)** indicated that juvenile Korean rockfish (20g) required 24h after the first feeding to empty their stomach.

A higher feed intake (dry basis) (Table,2) was observed in sea bass (*Dicentrarchus labrax*) that fed mixed dry pellet and trash fish (feeding schedule 4) in comparison to those fed on single diets (dry pellet or trash fish). This outcome suggests its value as a potential diet for sea bass (*D. labrax*) culture, may be due to related to adequate palatability or to an effort to compensate lipid and amino-acid deficiencies relative to fish **(Garcia Garcia and Cerezo Valverde, 2006; Cerezo Valverde *et al*.; 2008, 2009; Estefanell *et al*., 2011).** Though, the combination of dry pellet and trash fish (feeding schedule:4)have improving in feed efficiency (Table 3) and maximized growth rates (Fig.1) in *D.labrax*, suggesting that mixed diets may better cover the nutritional requirements of sea bass (*D.labrax*) **(Cagnetta and Sublimi, 1999).**

With respect to nutrient utilization, sea bass (*D.labrax*) that fed on mixed diets (dry pellet and trash fish, FSI) have highest energy intake (33.29kj/as dry matter) (Table,2) showed the highest values of feed conversion efficiency (FCE) (59.38%) (Table,3), energy retention (18.59%) (Table,4), protein efficiency ratio (1.11) (Table,3) which demonstrates the use of lipids as energy source.

Garcia Garrido *et al.* (2010)and **Estefanell *et al.* (2011)** with *Octopus* ***vulgaris*** showed that the use of lipid in *O.* ***vulgaris*** as an energy source during starvation. In this sense, lipid digestibility in *O.vulgaris* depends on the quantity and quality of dietary lipids **(Sánchez *et al*., 2009; Seica Neves *et al*., 2010).**

Analysis of variance showed that the differences in dry feed intake(as dry basis)for *D. labrax* fed only dry pellet diet 153.26g (Treat 1) and fish fed trash fish only 153.77g. (Treat 6) were insignificant ($P > 0.05$). This is may be due to the fact fish feeding behavior **(Xu *et al*., 2007).** The authors showed that *Epinephelus coioides* (120±14g) *Rhabdosargus sarba* (147±8g) tended to ingest soft tissues and spit out food containing bones, when fed trash fish, minced by scissors into pieces of 1-2cm. Though, they showed that feeding with trash fish may affect protein synthesis, digestion, food molecules breakdown in comparison with using other feeds. Since the chemical composition of trash fish was similar to that in other feeds. But the reduction should be identified.

Ng *et al*. (2000); Webster *et al*., (2002) reported that feeding rate and feed consumption in relation to body weight decreases as fish grow. In the current work, growth rate of European sea bass fingerlings fed 2.0 to 3.0% body weight/day at 22C° were appeared to be close to optimum **(Russell *et al*., 1996). Mihelakakis *et al.* (2002)** found that percent feed efficiency decreases curvilinear with increase feeding rate. Though, under a condition of low feeding rate, fish tend to optimize their digestion to extract more nutrients more efficiently, thus decreasing the feed conversion efficiency **(Van Ham *et al*., 2003).** They explained by the lower energy and protein retention at satiation feeding when higher energy requirement for feed absorption at higher feed consumption.

The present **study** in (Table 3) show that with increase feed consume (dry basis) in FS.4 (Table 3) decrease feed conversion efficiency and protein and energy retention, when comparative it with (fish fed only dry pellet diet (DP)). **Partridge and Jenkins (2002)** found that high feed intake results in a low feed conversion ratio. Moreover, knowledge of the optimum feeding rate is important not only for promoting best growth and feed efficiency, but also for preventing water quality deterioration as a result of excess feeding **(Ng *et al*., 2000; Webster *et al*., 2002).**

The food conversion ratio presented in the following descending (performed better) order: treatments (DP, FS.1, FS.2, FS.3, FS.4, TF respectively) (Table 3). In terms of food conversion ratio, **Tubongbanua (1987)** found that the pasteurized trash fish diet still performed better as indicated by low FCR compared to the rest of the trash fish diets. This may be due to the fact that trash fish had less enzyme thiaminase and contained higher levels of nutrients compared to the rest of the diets. The same **author**, showed that with regard to fish meal diets, the mashed fish meal gave a lower FCR as compared with the pelleted feed. It was observed that the fish would not feed on a hard and dry pellet but would eat the pellet once it had sufficiently soaked and absorbed water. The leaching out of the nutrients, during the time it stayed in the water could be the reason for the high FCR **(Tubangbanua, 1987).** Similar findings in the present study were noted the high FCR with treat (6 TF) sea bass (*D. labrax*) fed only trash fish (Table,3), this may be due to the trash fish was homogenized by grinding (it with a meat grinder) and diffusing and lost during the time, it stayed in the water. However, the unfavorable response to dry pelleted feed has also been observed by **Avance (1984).**

As expected **Shapawi *et al*. (2011)** found higher level of methionine and lysine were observed in trash fish (4.0 and 8.6g/100g amino acid, respectively) compared to the pelleted feeds on a dry weight basis. However, it should be noted that more than 70% of the weight of trash fish is made up of water.

The trash fish used in the present study (Table,1) contained high levels of protein (61.63% of dry basis). Nevertheless, *Dicentrarchus labrax* fed only trash fish (Treat 6, TF) had poor feed conversion ratio (1.75) compared to fish fed the pelleted feed only (Treat 1, DP) which a FCR (1.64 respectively) which were significantly better (Table, 3). A similar trend was reported by **Shapawi *et al*. (2011)** who noted that the poor FCR of groupers fed trash fish is due mainly to losses of feed material during feeding. Unlike pelleted feed, trash fish break up into small pieces when eaten. Consequently as much as 30 to 50% of the trash fish fed to fish can be lost during feeding process **(Sim *et al*.,2005). Rimmer,(2004)**and **Orchunwong *et al*. (2005)** show that despite the high nutritional content of trash fish, poor FCR values observed in trash fish feeding is common in marine fish culture. In this connection, **Navas *et al*. (1998)** reported that the trash fish profile was 710g Kg^{-1} Protein and 23.2% DHA. Also, **Shapawi *et al*. (2011)** found that the trash fish contained high levels of protein (701 g Kg^{-1}) and DHA (21.5% of total fatty acid).

The reported food conversion ratio, FCR (expressed in dry basis) for European sea bass fed only trash fish (Table,3) were incease (1.75) than European sea bass fed only dry pellet diet (1.64). On the other hand, **Cardenete *et al*. (1997)** reported that FCR (expressed in dry bassis) for fresh food (Mixture composed of 50% fish (bogue) and 50% squid) when feeding *Dentex dentex* were better than fish fed commercial pellet diets. This fact seems to indicate a difference in the quality of the dietary components. The same authors, found that fish fed with fresh food was highest of protein/energy ratio of the diet (39.9) than dry pellet diet (26.1-27.7), the protein clearly being used as a source of energy rather than for weight gain as reported in other fish species (for red sea bream **Takeuchi *et al*, 1991;** for gilthead sea bream, **Vergara *et al*., 1993).** Though, this may have been due to higher carbohydrate content of dry pellet diet than with fresh food **(Cardenete *et al*., 1997).** In addition, it is worth mentioning that fish fed the dry pellet diet displayed discolouration by the end of the experiment, as compared with fish fed the fresh food diet.

The present **investigation** (Table,3) showed decrease feed efficiency for sea bass (*D. labrax*) fed only artificial diet than previous research, may be due to **presence of** some ingredient like meat and bone meal which is low protein digestibility coefficients. **Zhou *et al*. (2004)** showed that the apparent phosphorus digestibility in juvenile cobia of meat and bone meal was the lowest may be to highest phosphorus in meat and bone meal, which agrees with results reported for rainbow trout, turbot and Atlantic Salmon **(Burel *et al*. 2000).** In this connection **Lall (1991), recorded** that phosphorus present in phytate is Known to be unavailable to fishes due to the lack of endogenous or microbial phytase in their intestinal tract, which is required to digest phytate. Though, **Zhou *et al*. (2004)** reported that the lower apparent digestibility coefficient values of phosphorus, may be due to its higher glucosinolates content. **Opsvedt *et al*. (1984)** appeared that meat and bone meal had a lower availability coefficient for lysine. This could indicate heat damage to lysine during the rendering process or possibly reduced digestibility of protein in bone fragments.

In Table,**(**3) **it was** indicated that treat (FS.4) gave improved in protein efficiency (1.11) and protein retention (21.54) (Table 4) than other treat ((FS.1; FS.2; FS.3, TF) fed mixed pellet and trash fish. However, analysis of variance showed that the differences in PER and PR were insignificant ($P > 0.05$) between treat (1 DP) fed pellet dry feed and treat 5 (FS.4) fed mixed (pellet dry feed and trash fish) (Table 3,4). It is clearly apparent that the quality of ingested protein for *D.labrax* fed mixed (dry pellet feed and trash fish: treat 5, FS.4) may be the cause of improved protein efficiently ratio in this treatment.

A similar **trend** was reported by **Yigit** ***el al.*** **(2003)** who found that the protein utilization improved and less protein was excreted as ammonia-N when fish (Black sea trubat) were fed goby (*Gobius Sp*.) or whiting (*Merlangius marlongius*). Also, this finding is in agreement with **Eid and Matty (1989),** for **carp** and **Ballestrazzi** ***et al.*** **(1994),** for *D. labrax*, who reported that the ammonia excretion as a percent of ingested nitrogen depends on the composition and quality of the diet. In this regard, **Warren and Davis (1967)** showed that the quantity and quality of the feed, the metabolic state of the fish, and the energy demands for maintenance and activity will largely determine the effect of food consumed on growth.

Poor nutrient utilization in the present study for sea bass *(D. labrax)* fed only trash fish (Table,3) may be due to low in protein quality **(Tantikitt,** ***et al*****., 2005).** However, **Lam,** ***et al.*** **(2008)** showed that most fish farmers in Malaysia are still using minced trash fish because of its low price and convenience. The same authors, **added** that total suspended solid. are uneaten feed (spitted out from the fish's mouth during experiment) (TSS) produced from marble goby represented approximately 9-13% of the daily feeding rate.

Sanchez –Muros ***et al.*** **(2003) showed** that better diet utilization when fed at certain times of the day could be explained by coincidence with natural rhythms of secretion, activation or synthesis of digestive and/or metabolic enzymes. The intestinal protease activity in European sea bass has been described as having a nychthemeral rhythm with increased activity at night, regardless of the frequency of feeding as deprivation **(Martinez-Bebia** ***et al*****., 1995). Boujard and Leatherland (1992)** found that the adaptation of the feeding schedule to these metabolic, digestive and other rhythmic processes related to the utilization of nutrients (i.e. hormone release) appears to improve FCR and PER indices when the fish are fed according to their natural rhythm of ingestion. Knowledge of the energy and protein content of the weight gain of fish allows an estimate of the necessary dietary supply **(Lupatsch** ***et al*****., 2001).**

In the present trials, **regarding** body composition (Table 4) displaying narrow limits for protein (protein retention (17.85 and 23.74%), but wide ranges for lipid deposition (lipid retention, 11.7 and 18.18%). This finding agrees with that reported by **Lupatsch** ***et al.*** **(2001).** With gilthead seabream (*Sparus aurata* L.) the same authors reported that there seems to be a common pattern where fish tend to increase their lipid deposition with increase fat levels in diets in conjunction with decrease protein intake. As stated previous with *S.aurata* **(Lupatsch** ***et al*****., 1998)**, the protein level stayed at a constant whereas the lipid level increased with increasing fish size. Some discrepancies was found in the present study which show that sea bass (*D.labrax*) fed only dry pelletet diet (DP) it have lipid deposition higher (3.45%) than fish fed only trash fish (TF) (3.0%) (Table,4) may be due to conjunction with decreasing protein intake in Treat,1 (DP) (75.54) than Treat 6 (TF) (94.46g), in spite of decrease fat levels in dry diet than trash fish (Table,2). These, may be also due to decrease P/E ratio 25.86 in dry pellet diet than P/E ratio (28.30) in trash fish (Table,1).

On the other hand, **Lupatsch** ***et al.*** **(2001)** found that, **in** *Sparus aurata*, the relationship between daily digestible energy (DE) intake and energy retained per Body weight $(Kg)^{0.83}$ was found to be linear, and thus efficiency of utilization of DE was constant as demonstrated previously **(Lupatsch** ***et al*****. 1998).** Also, the diet **was** deficient in nonprotein energy, protein will be used for energetic purposes rather than for protein synthesis, causing reduced growth even with high dietary protein content, and hence resulting in lower efficiency **(Lupatsch** ***et al*****. 2001)**. A similar trend was found for show deficient of non protein energy when fish fed only trash fish (Treat.6, TF) (Table,2) which causing reduced nutrient utilization than sea bass (*D. labrax*) fed only dry pellet treat (1, DP) (Table,4). *D. labrax* fed the only trash fish (Treat. 4) exhibited a lower significantly ($P < 0.05$) in lipid deposition (3.00%) and fat retention (11.71%) than other fed schedule (Table 4). A possible explanation is a more efficient utilization of dietary lipids (lipid consume was higher significant than other fed schedule) as energy substrates (25.64g dry-basis) as shown by a lower fat retention and body fat content (**Company** ***et al*****., 1999)**.

The value of fat and energy retained (Table 4) were related to fish meal (dry basis) consume for European sea bass of all groups (Treat 1 to 6) (Table). These results reflecting high fat and energy retained with decrease level of fish meal (dry basis) consume. A similar tendency was also observed in *Dicentrarchus labrax*, with increasing levels of fish meal replacement, the high fat and energy retention values (**Kaushik *et al*. 2004)**. The same authors suggested that there was increased lipogenesis with increasing levels of fish meal replacement by plant protein.

The high deposition of body fat (Table 4) indicated that the dietary lipid level (lipid consume) in treat (1) was not utilized by the fish for energy (**Shapawi *et al.* 2011**).

However, in the present study, the highest protein efficiency ratio (1.23) (Table,3) (treat,1, DP) did not concur with maximum growth for treat.5, FS.4 (Fig.1). The same pattern was observed by **Santinha *et al.* (1996)** where a 55% protein diet gave the best growth in gilthead sea bream (9and 63g), but a 40 protein diet showed the highest protein efficiency. These finding were agrees with reports in other fish species such as tilapia **(Kaushik *et al.* 1995)** and trout **(Kim and Kaushik, 1992).**

In the present study, **the** results revealed (Table,4) that energy retention increased significantly ($P < 0.05$) with fish fed (mixed dry pellet feed and trash fish, treat.5 Fs.4). 18.59% ± 1.42 than other treat fed mixed diets (Fs.2; Fs.3, TF) (17.97, 16.93; 15.66% respectively). This may be due to increased dry feed intake for treat fish (5) (166.28g) than other treat fish 3, 4, 6 (157.79, 154.99, 153.27g respectively) (Table 2). A similar trend was found by **Mihelakakis *et al* (2002)** with *Sparus aurata,* and **Jobling (1994)** who showed the proximate composition of fish varies with food quality and quantity with lipid deposition tending to increase with increased food supply. Though, **Mihelakakis *et al.* (2002)** suggested that fish fed maximally will generally contain proportionally more lipid than those fed restricted rations.

Histological observation in intestine of *Dicentrarchus labrax* (Plate 1: a,b,c,d,e,f) **revealed the** reaction to fed dry pellet diet or trash fish with four feeding schedule. The degenerative changes included fused micrvilli, the outer membrane of microvilli are broken cell swelling and hemorrhage in the submucosa regain with increased feed intake (Table 2) from dry pellet diet comparatively to fish feed consume from local trash.

When discussing the inclusion of both trash fish and formulated diet alone or inside feeding schedule in dietary European sea bass*, D.labrax,*(Plate 1), it is of high importance to evaluate the effect on gut histology by light microscopy, because structural and functional changes in the intestine may explain deleterious effect of feeding schedule on nutrient utilization and disease resistance **(Harper *et al.*, 2011)**. **Raskovic *et al.*(2011)** show that histopathological changes in the intestine may vary depending on the species and feed used in the experiments. In fish from *Salmonidae* family, the replacement of fish meal protein with plant protein cause reduced growth rates and pathological changes, particularly enteritis in the distal part of the intestine **(Uran *et al*,. 2009).**Though, in common carp (*Cyprinus carpio* L.), **Uran *et al*., 2008)** found a short – term reaction, and inflammation to soybean, but after a period of month adaptation, the intestine returns to a normal histological structure, as well as an increase in the number of mucous cells in rainbow trout (**Poleksic *et al*.**,2006). However, **Kowalska *et al.*,(2011)** observed larger and more numerous lipid vacuoles and lipid droplets in the enterocyte and superanuclear vacuoles were confirmed in pikeperch *(Sander lucioperca)* fed feed supplemented with 100g lipid /kg diet, which concurs with present study (Plate 1) showed degenerated villi in all treatment, may be to increased feed intake of lipid (Table 2).

In this connection**, Martinez-Liorens *et al.* (2012)** reported that, the analysis of the histological parameters for the distal intestine of gilthead sea bream *Sparus aurata* revealed some differences among the fish fed with different diets. Histological, the distal intestine consists of three layers; the serous layer (SL), the muscular layer (ML) and the mucosa layer (ml). The mucosa layer includes the mucosal epithelium, lamina propria (Lp) and submucosa layer (SBL).As well as, histological changes have also been reported in the distal intestine of Atlantic salmon fed diets containing soybean meal **(Baeverfjod and Krogdahl, 1996)**. This is important for fish growth, because although distal intestine is not an important site for nutrient absorption in Salmonids, still absorption of both protein and fat in the distal intestine has been suggested **(Ezeasor, 1978)** and is the region of the intestine where alterations are commonly observed. Similarly, intestinal changes associated with high dietary vegetable protein inclusion have been found in Asian sea bass (*Lates colcarifer*) and Atlantic Salmon **(Boonyaratpalin *et al*., 1998**). Though five phases were established during the development of the digestive tract of *Sparus auratus* in accordance with anatomical and histological features:Newly hatched (phase I),2-3 days after hatching (phase II),4-7 days (phase III),8-59 days (phase IV) and 60-69 days (phase V), while in a previous investigation only endotrophic,endo-exotrophic and exotrophic phase were described **(Calzada *et al*.,1998).** Most teleosts pass through a larval period in which a completely differentiated stomach appears several weeks after the start of exogenous feeding **(Ribeiro *et al*.,1999)**. In *S. auratus*, a presumptive stomach can be recognized 3 days after hatching and is completely developed after 69 days, when the gastric glands are present through. The first gastric glands are frequently found during the transition period form larvae to juvenile, when complex digestive processes are required to assimilate new foods in a new habitat **(Pedersen and Falk-Petersen, 1992)**. In *Dicentrarchus labrax*, the first pyloric caeca were found before the gastric glands appeared **(Garcia Hernandez *et al*., 2001).**The gastrointestinal (GI) tract of fish is considered as one of the major infection routes for some pathogens and indigenous intestinal bacteria **(Ringo *et al*.,2010).** Indeed, numerous studies have reported that exposure of the epithelium to fish pathogens can result in sever tissue damage, characterized by necrotic enterocytes, deteriorated tight junctions; disorganized and damaged microvilli and damage to the lamina propria **(Ringo *et al*., 2007).**

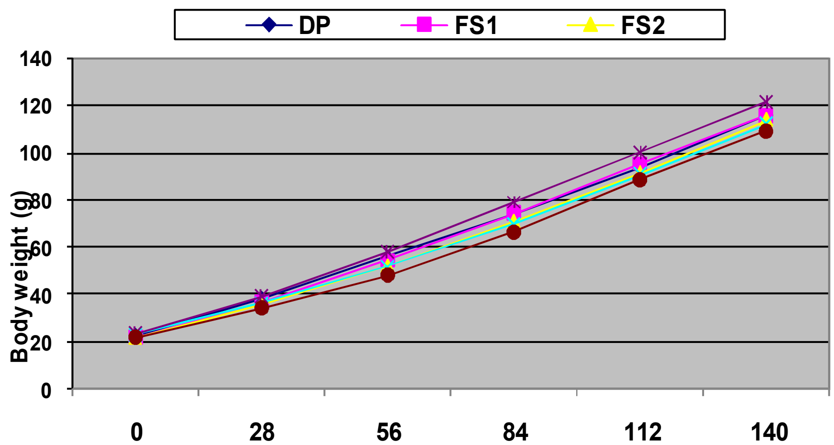

Fig. (1) Mean daily body weight of *Dicentrarchus labrax* fed at different feeding schedules

Table (1). Ingredients and proximate composition of experimental diets

Ingredients (g/100 wet matter)	**Diets**	
	Dry pellet	**Trash fish**
Trash fish	-	100
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 + sun flower oil (1:1)	5.0	-
Vitamin [1]	1.5	-
Mineral [2]	1.5	-
Proximate composition		
Moisture (%)	9.23	74.54
Crude protein (% DM) [3]	49.29	61.63
Crude fat (% DM)	12.33	16.73
Crude ash (% DM)	17.14	19.4
Crude fiber (% DM)	7.78	-
Nitrogen-free extract (% DM)	13.46	2.24
Gross energy [4] (Kjg^{-1})	19.06	21.78
Protein energy ratio (P/E) (mg protein/kj)	25.86	28.30

1- 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).

2- 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).

3- DM: Dry matter

4- Gross energy (kjg^{-1}): Calculated using = 23.9Kjg^{-1} protein; 39.8kjg^{-1} lipid; 17.6kjg^{-1} carbohydrates (cited by, **Martinez-liorens *et al*., 2007**).

Table (2) Feed consume (g) of European sea bass (*Dicentrarchus labrax*) raised on two type of diets (dry pellet (Dp) and trash fish (Tf)) and four feeding schedule (Fs).

Diets and feeding schedule Feed consume (g/fish) as dry matter	Dry pellet diet (DP)	Feeding schedule.1		Feeding schedule.2		Feeding schedule.3		Feeding schedule.4		Local trash fish (TF)
		Dp	Tf	Dp	Tf	Dp	Tf	Dp	Tf	
Feed consume as fed	168.84±7.5	84.0	328.31	85.53	314.80	53.48	418.10	118.32	231.26	602.01±25.77
Feed consume as dry matter	153.26±6.8	76.25	83.58	77.64	80.15	48.54	106.45	107.40	58.88	153.77± 6.56
Total feed consume as dry matter	153.26±6.8	159.83±5.9		157.79±1.1		154.99±5.8		166.28±6.6		153.77±6.56
Protein intake	75.54±3.35	37.58	51.51	38.27	49.40	23.93	65.61	52.94	36.29	94.46±4.1
Total protein intake	75.54±3.35	89.09±3.27		87.67±4.1		89.54±3.23		89.23±3.70		94.46±4.1
Protein intake from fish meal	40.76±2.7	71.78±3.10		70.04±3.7		78.52±2.8		64.85±2.9		94.46±3.4
Lipid intake	18.90±2.03	9.40	13.98	9.57	13.41	5.98	17.81	13.24	9.85	25.64±2.43
Total lipid intake	18.90±2.03	23.38±1.7		22.98±2.21		23.79±1.93		23.09±2.06		25.64±2.43
Total lipid intake from fish meal and oil	9.51±1.45	18.71±1.02		18.22	1.51	20.82±1.22		16.51±1.13		25.64±2.43
Carbohydrate intake	20.63±2.14	9.33	1.87	9.50	1.80	5.94	2.38	13.14	1.32	3.43±0.72
Total carbohydrate intake as dry matter	20.63±2.14	11.20±1.61		11.30±1.98		8.32±1.24		14.46±1.69		3.43±0.72
Fiber intake	11.92±1.06	6.98±1.18		6.98±1.14		4.37±0.81		9.67±1.13		-
Energy intake (kj)	29.21±2.75	14.53	18.20	14.80	17.46	9.25	23.18	2047	12.83	33.38±2.73
Total energy intake (kj)	29.21±2.75	32.73±2.9		32.26±1.85		32.43±2.07		33.29±2.65		33.38±2.73
Ash intake	26.27±3.6	13.07	16.21	13.31	15.55	8.32	20.65	18.40	11.42	30.24±2.51
Total Ash intake	26.27±3.01	29.26±2.78		28.86±2.23		28.97±1.93		29.83±2.31		30.32±2.5
Protein to energy intake %	25.86±1.19	27.22±0.31		27.18±1.09		27.61±1.28		26.80±1.33		28.30±1.26
Carbonydrate/proein intake ratio	0.273	0.126		0.129		0.093		0.162		0.036

Values are means ± S.E of two replicate of net pen culture. Mean with different superscript letters with in rows are significantly differently ($P < 0.05$).

Table(3): Feed efficiency of *Dicentrarchus labrax* fed the dry pellet (DP) and local trash fish (TF) with different schedule (Fs)

Diets and feeding schedule	Feed consume as dry matter	Feed conversion ratio	Feed conversion efficiency	Protein efficiency ratio	Lipid efficiency ratio	Energy efficiency ratio	Carbohydrate intake / protein intake as dry bass (%)
Dry pellet diet (DP)	153.26±6.8	1.64±0.29	60.81±3.13 ^{a}	1.23±0.03	4.93±0.36 ^{a}	3.19±0.33 ^{a}	27.3±2.84 ^{a}
Feeding schedule, 1 (FS1)	159.83±5.9	1.70±0.32	50.89 ±2.77 ^{b}	1.06±0.046	4.03±0.51 ^{ab}	2.88±0.27 ^{b}	12.6 ±1.37 ^{bc}
Feeding schedule. 2 (FS2)	157.79±7.1	1.71±0.27	58.36±2.52 ^{a}	1.05±0.027	4.01±0.39 ^{ab}	2.85±0.23 ^{b}	12.9 ±1.46 ^{b}
Feeding schedule. 3 (FS3)	154.99±5.8	1.73±0.23	57.68 ±3.11 ^{a}	1.00±0.015	3.76 ±0.42 ^{b}	2.76±0.31 ^{b}	9.2 ±0.87 ^{c}
Feeding schedule. 4 (FS4)	166.28±6.6	1.68±0.19	59.38 ±3.42 ^{a}	1.11±0.021	4.28 ±0.49 ^{a}	2.97±0.28 ^{ab}	16.2 ±1.52 ^{b}
Local trash fish (TF)	153.27±6.56	1.75±0.24	57.04 ±2.26 ^{a}	0.93±0.34	3.41 ±0.31 ^{b}	2.62±0.25 ^{c}	3.6 ±0.71 ^{d}

Values are mean ± standard error of two replicates. Values in a row with the same letter are not significantly different ($P < 0.05$).

Table (4): Daily feed intake and nutrient utilization of European sea bass (*Dicentrarchus labrax*) raised on two types of diets (dry pellet (DP). and trash fish (TF) and four feeding schedule (Fs).

Diets and feeding schedule	Dry pellet diet (DP)	Feeding schedule.1	Feeding schedule.2	Feeding schedule.3	Feeding schedule.4	Local trash fish (TF)
Relative feed intake (%/day) as dry matter	1.64 ±0.071	1.72±0.056	1.73±0.06	1.70±0.011	1.70±0.08	1.72±0.07
Daily feed intake of local trash fish as dry matter (%/day)	-	0.893±0.02	0.871±0.04	1.161±0.05	0.598±0.02	1.72±0.07
Protein deposition (g)	a 17.93±1.68	18.11 ±1.57	ab 17.76±1.38	b 17.35±1.49	a 19.22±1.85	b 16.86±1.34
Lipid deposition (g)	ab 3.45±0.80	3.99 ±0.63	a 3.82±0.55	b 3.38±0.61	a 4.00±0.49	b 3.00±0.52
Energy deposition (Kjg^{-1})	ab 5.655±273	5.927 ±0.98	a 5.797±1.72	b 5.490±1.03	a 6.189±1.13	b 5.227±0.86
Protein retention (%)	a 23.7±2.15	20.33 ±2.05	ab 20.26±1.79	b 19.38±1.71	a 21.54±2.19	b 17.85±1.56
Lipid retention (%)	a 18.18±1.35	17.07 ±1.29	b 16.62±1.53	b 14.21±1.29	a 17.36±1.40	c 11.71±1.12
Energy retention (%)	a 19.36±1.75	18.11 ±1.32	ab 17.97±2.02	b 16.93±1.58	a 18.59±1.31	b 15.66±1.15

Values are mean ± standard error of two replicates. Values in a row with the same letter are not significantly different ($P < 0.05$).

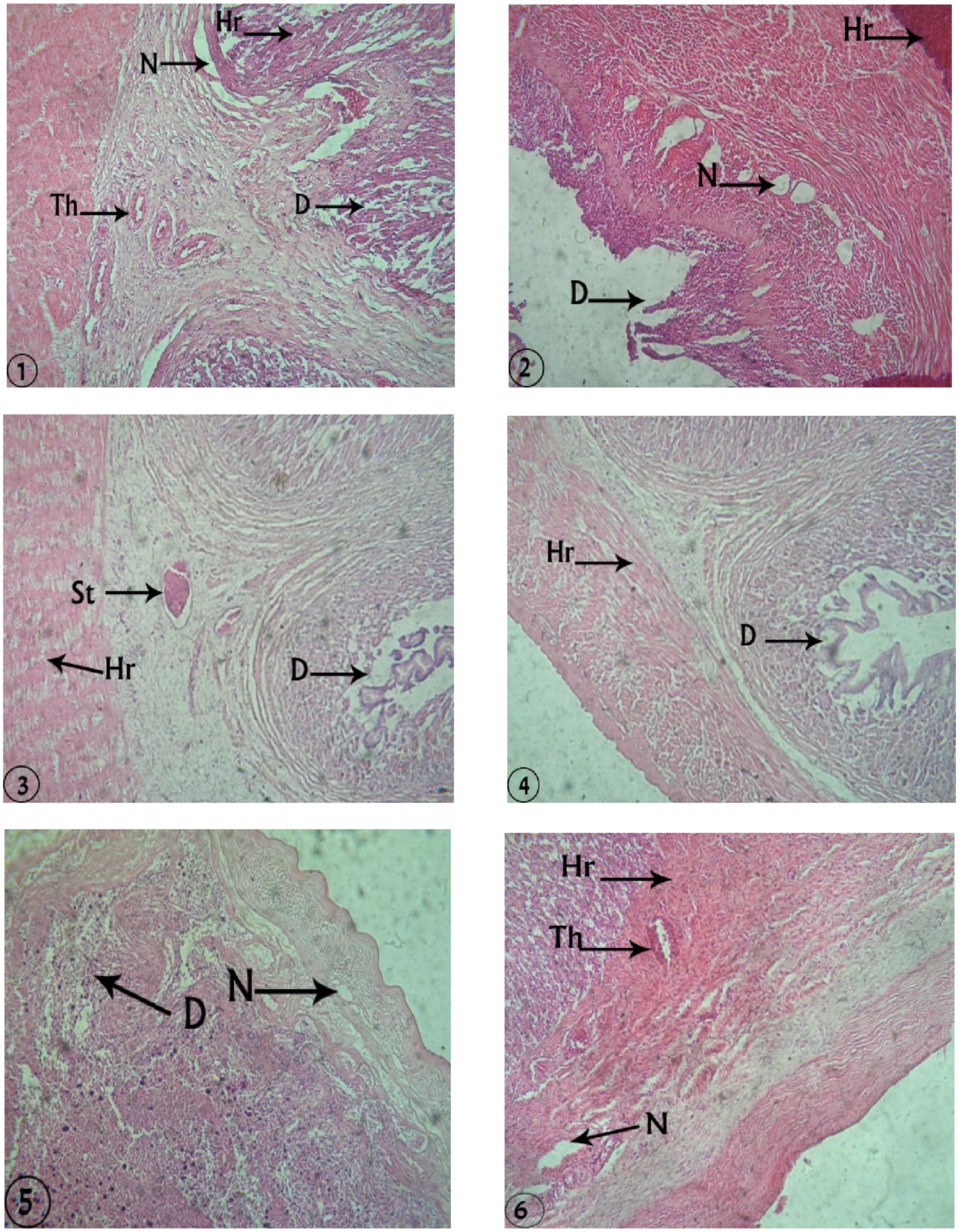

Plate (1).Intestine

Histological observation in intestine of *Dicentrarchuslabrax*

(Fig: 1,2,3,4,5,6) reaction to fed dry pellet diet or trash fish with four feeding shedule: fused microvilli, the outer membrane of microvilli are broken cell swelling and hemorrhage in the submucosa region (Hr),with increased feed intake,degenerated villi (D) in all treatment may be to increased feed intake of lipid. Stagnant blood (St). Necrosis (N). Thick wall of blood vessels (Th).

In this connection, **Kraugerud *et al*.(2007)** observed morphological changes, on Atlantic salmon, in the distal intestine of fish fed 10% dietary defatted soybean meal for 4 weeks. These changes included shortening and fusion of the simple mucosal folds, widening of the lamnia propria with increased cellularity, leucocytic cellular infiltration of the submucosa and lamina propria and reduced supranuclear vacuolization with apial nuclear displacement within enterocytes. Though, **Merrified *et al.* (2009)** observed the effect of replacing 50% of fish meal with soybean meal (Hipro soya) on the intestinal epithelium of rainbow trout. They found that fish fed the soybean meal enriched diet displayed missing, damage, deformed, shorted and thicker microvilli. The same authors showed that this reduction of microvilli density consequently led to increased exposure of enterocyte tight junctions, which combined with necrotic enterocytes likely to diminish the protective barrier of the intestinal epithelium. On the other hand, the most predominant effect of increasing plant oils in the diets marine fish, is the reduction of cellular essential fatty acids content and some alteration in membrane composition, also alter the ratio of n-6/n-3 C2o PUFA, which may have pronounced effect on immune functions and eicosanoid production with the inclusion of pant oil into aquafeeds, fish are faced with several challenges some of which will affect intestinal health and function. One of the most striking effects is the massive accumulation of lipid droplets in enterocytes of fish species such as gilthead sea bream **(Caballero *et al.*, 2003)**. This accumulations may amount to more than 60% of cellular volume which hampers gut functions. **Sire and Vernier (1992)** showed that the contact surface between absorbing epithelium and intestinal contents can be increased by stretching the segment and / or by the formation of folds, occasionally accentuated in the form of a posterior annulo-spiral as in the *Salmonidae,* and /or by the presence of more or less numerous anterior caecae. A basic character shared by all teleost fish is the presence of at least two intestinal segments.The first assures the absorption of lipids **(Sheridan,1988)** and the second is responsible for the pinocytotic uptake of macromolecules **(Gas and Noaillac – Depeyre, 1981).**

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