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EFFECT OF REPLACING FISH MEAL PROTEIN BY PLANT PROTEIN SOURCES, STOCKING DENSITY AND CONCRETE PONDS AREA ON GILTHEAD SEA BREAM (*Sparus aurata* L.) FRY PRODUCTION.

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SUMMARY

Two trials were conducted with fry gilthead Sea bream, the first studied the effect of partial replacement of fish meal by plant protein (PP) (33%, 50% and 66% PP) under two stocking densities (40 and 55 fry/m^3). The second was to study the effect of concrete pond area (1 and 10 m^3 water capacity). The results showed that in the first trial, the partial replacement of fish meal protein with plant protein had significant effects (P≤0.05) on the growth performance parameters such as final weight, total gain and specific growth rate. While the highest values were obtained with the diet contained 33% plant protein. Survival rate was within the range 83.55-86.58% with significant differences. The higher feed consumption was observed for those fish fed higher levels of fish meal (33% PP) under density 55 fry/m^3 compared to other diets. Regarding to docking density the results indicated that 55 fry/m^3 improved growth performance and FCR compared with stocking density of 40 fry/m^3. Diets affect body chemical composition significantly where 50% PP had the greatest effect of body protein and the lowest ether extract. In the second trial, results showed that concrete ponds area had significant effects (P≤0.05) on growth performance parameters and improved FCR where big pond record (10 m^3) highest values.

Keywords: *gilthead sea bream, growth performance, feed utilization, chemical composition, stocking density, plant proteins,*

INTRODUCTION

Marine fish farming in Egypt began in 1976 with the culture of gilthead sea bream, (*Sparus aurata*) as this fish was notably adaptable to brackish and marine pond conditions (Sadek, 2000). Gilthead sea bream are widely distributed throughout the mediterranean countries and the production rate increased from 2003 to 2007 by 109% in Egypt (Yeroulanos, 2010). Egypt production was 2884 tonnes in 2007, representing 39 % of sea bream world production, (FAO 2009).

Fish meals provide dietary protein with essential amino acid profiles similar to dietary requirements. However, much work is being done to reduce its proportion in commercial diets by partial substitution with various alternative animal and vegetable protein sources, in order to guarantee the supply of high quality diets to fish farmers, avoiding availability, quality and costs oscillations associated with fish meal (Tacon and Jackson, 1985).

Among plant protein sources, some studies reported that partial substitution of dietary fish meal with corn gluten meal (12 to 26% of the diet) has lead to satisfactory results of growth rates and feed utilization in diets for rainbow trout (Moyano *et al.*, 1992).

Gomez-Requeni *et al.* (2004), Vega-Rubin *et al.* (2004) and Sitja-Bobadilla *et al.* (2005) studied the effect of partial or total replacement of fish meal (FM) by a mixture of plant protein (PP) sources (corn gluten, wheat gluten, extruded peas, rapeseed meal and sweet white lupin) in juvenile gilthead sea bream diets. Final body weight was progressively decreased with PP inclusion. However, at the end of the trial, specific growth rates (SGR) were reduced only by 2.4% and 4.2% in 50% PP and 75% PP groups and their feed efficiency (FE) was significantly improved in comparison with fish fed the FM diet. At the highest replacement level (100% pp), SGR was highly reduced (21.6%) and feed efficiency was not changed with respect to FM fed fish. They concluded that it is possible to replace fish meal by PP without any adverse effect on gilthead sea bream growth and feed conversion ratio.

On the other hand Santigosa *et al.*, (2011) found that final body weight and feed intake decreased progressively and significantly with the increase of dietary plant protein content. Conversely, feed and protein efficiency values were high in fish fed plant protein diets, and the best protein efficiency ratio was found in fish fed the 75% PP diet.

De Francesco *et al.* (2007) studied the effect of diets either based on fish meal (diet FM) or on a mixture of plant protein sources such as corn gluten meal, wheat gluten, extruded peas, rapeseed meal and extruded whole wheat (diet PP), replacing 75% of fish meal protein. Feed intake was higher in sea bream fed diet FM (0.48 versus 0.44), while feed efficiency and protein efficiency ratio were significantly higher in sea bream fed PP (0.83 versus 0.77 and 2.0 versus 1.76, respectively). The whole-body composition was similar between the two sea bream groups and no differences were observed.

Stocking density is one of the most important parameters affecting the growth, performance, and productivity in fish farming activities. A specific stocking density can have either positive or negative effects on fish growth, and this interaction seems to be species-specific (Merino *et al.*, 2007).

Crowding is a factor involved in physiological stress and responsible for the increase in plasma cortisol, which plays an important role in the low efficiency of immunological responses under these conditions (Barton, 2002; Mommsen *et al.*, 1999 and Di Marco *et al.*, 2008). Some studies showed the increase in stocking densities improved growth performance parameter in gilthead sea bream (Yilmaz and Arabaci 2010), in sea bass (Papoutsoglou *et al.*, 1998 and Sammouth *et al.*, 2009) this improved related to the immunological responses and physiological processes, mainly those related to metabolism and behavior (Vijayan *et al.*, 1990; Irwin *et al.*, 1999; Barcellos *et al.*, 2004; Kristiansen *et al.*, 2004; Schram *et al.*, 2006). In gilthead sea bream, *Sparus aurata*, different stocking densities altered fatty acid metabolism, with a decrease in hepatic oleic acid, a monounsaturated fatty acid important as energy source, mainly in higher stocking densities (Montero *et al.*, 1999).

Stress in farmed fish is of considerable significance to both welfare and productivity as it has been linked to reduction in growth, abnormal behavior and immuno-depression (Wedemeyer, 1997; Ashley, 2007). Particular attention has been drawn to stocking density as one of the key factors to influence the perceived level of stress in fish (Ellis *et al.*, 2002; Turnbull *et al.*, 2005; North *et al.*, 2006). Higher stocking density increased the response to an acute stress or, however this effect was dependent on feeding level and significantly reduced in well fed fish (Lupatsch *et al.*, 2010).

Pond area also raise growth rates in terms of increased space leads to increase in growth rates in gilthead sea bream (JICA 2010) and in tilapia (Abouzied and Hassouna 2006)

This study was conducted to investigated the effects of partial replacement of fish meal protein with plant protein source, two stocking density and concrete pond area on the growth performance, feed utilization and body composition of fry sea bream.

MATERIALS AND METHODS

Experimental design

Gilthead Sea bream (*Sparus aurata L.*) fry were obtained from a private hatchery in Ismaelia governorate. Fish were acclimated to laboratory conditions for 14 days before being randomly distributed into concrete ponds of 1 m^3 and 10 m^3 water capacity in two trials, in National Institute of Oceanography and Fisheries (NIOF), Shakshok fish research station, El Fayoum Governorate. The water was obtained from Lake Qaroun. Water temperature ranged from 28 to 33°C and water salinity ranged from 29 to 32 ‰. Oxygen concentration was suitable (5.3-6.8 Mg/L) and pH ranged from 7.07 to 7.55. Fish of 0.21 ±0.02 g initial body weight were used.

Diets and stocking density

Fish were fed on three diets where the animal protein source was substituted by 33%, 50% and 66 plant protein (PP) source (mixture of corn gluten, yellow corn and soybean meal) and two stocking densities (40 and 55 fry/ concrete pond) twelve experimental concrete ponds of 1 m^3 water capacity each were used, in duplicate groups. The diet were formulated to contain about 45% crude protein (Table 1). Diets were free of amino acids and were hand made. During the growth trial, each diet was randomly allocated to duplicate concrete ponds of fish. Trial began 20/ 6/ 2011 and ended 18/ 9/ 2011 (90 days). Feed was offered by hand at three meals/day (8:00, 12:00 and 15:00 h) at 10% of body weight daily and the amount of diets were readjusted after each weighing.

ponds area.

Fish of 0.21 ±0.02 g initial body weight were allocated with 55 fry/ m^3 into two different concreat pond sizes (1 m^3 and 10 m^3).

Statistical analysis

The data were analyzed by one-way ANOVA and significant differences were determined by Duncan's Multiple Range Test at 5% level using SPSS Statistical Package Program (SPSS, 2010) 8.0, released version.

Table (1). Composition of the experimental diets used in the first Experiment.

Ingredients %	Diets		
	33% PP	50%PP	66% PP
Fish meal, (CP 71%)	43	32	22
Soybean, (CP 44%)	20	20	24
yellow corn	16	14	10
Corn glutin, (CP 60%)	8	21	30
Calcium phosphat	1	1	1
vit & Min. **	2	2	2
Linseed oil	10	10	11
Chemical composition	(as fed, %)		
Dry matter, DM	92.05	89.85	90.56
Crude protein, CP	45.73	45.52	45.18
Ether extract, EE	15.61	15.61	15.66
Crude fiber, CF	2.01	4.21	2.20
Ash	7.69	7.44	7.69
Nitrogen free extract. NFE[1]	21.01	17.07	19.83
GE, kcal/g*	4.999	4.916	4.932
Prices of Kg, L.E	11.05	10.59	10.46

1, Calculated by differences .

** Calculated according to NRC, 1993.*

*** According to Santigosa et al., 2011*

RESULTS AND DISSCUSIONS:

1- Effect of partial replacement of fish meal by plant protein sources (regardless stocking density effect) on:

1- Growth performance and survival rate of gilthead Sea bream.

Results of growth performance parameters and survival rate of fish fed with the three different diets are shown in Table (2).

The results cleared that the partial replacement of fish meal protein with plant protein had significant effects (P≤0.05) on the growth performance parameters such as final weight, total gain, daily gain and SGR. While the highest values were obtained by the diet contained 33% of protein from plant protein compared with other diets (50 and 66% PP). Survival rate was within the range 83.55–86.58%, and significant differences were observed. These results led to believe that the fish meal presented in the diet resulted in improving growth rate as it has better essential amino acid profile and a good source of essential minerals and vitamins. These results agreed with the results of Gomez-Requeni *et al.* (2004); Sitja-Bobadilla *el al.* (2005) and Vega-Rubin *et al.* (2004) who obtained significant differences with growth performance parameters in Sea bream fed plant protein source diets, compared with those fed fish meal diet. On the other hand these results disagree with Robaina *et al.* (1997) who found that no observed significant differences in growth in fish fed plant protein source diet (Corn gluten meal CGM) compared with those fed fish meal diet.

2- Feed utilization

The results in Table (3) showed that significant differences ($P \leq 0.05$) were obtained in all feed utilization parameters between treatments. Feed intake values were highest with diet containing (33% PP). The best FCR was recorded with diet containing (33% PP), where the worst FCR was recorded with diet containing (66% PP). Similar results were obtained for PER and EER, although values obtained for fish fed 33% PP diet were relatively higher than those fer 50 and 66% PP diets. PPV values were highest with diet containing (50% PP), but EPV values were highest with diet containing (33% PP). This may due to that Sea bream could digest and assimilate the FM protein in their bodies better than plant protein. The highest feed consumption values were observed by fish fed higher levels of FM. These results agreed with the results of Gomez-Requeni *et al.* (2004); Sitja-Bobadilla *el al.* (2005) and Vega-Rubin *et al.* (2004) but it differs from data obtained by Robaina *et al.* (1997), who reported that higher feed consumption was observed for fish fed higher levels of CGM compared with those fed fish meal diet.

Table (2). Effect of partial replacement of fish meal protein by plant protein sources on growth performance and survival rate of gilthead Sea bream.

Item	Diets			SED
	33% PP	50% PP	66% PP	
Initial weight (g/fish)	0.21	0.21	0.21	
Final weight (g/fish)	2.42^{a}	1.54^{b}	1.41^{b}	0.365
Total gain (g/fish)	2.21^{a}	1.33^{b}	1.20^{b}	0.365
Daily gain (mg/d)	24.56^{a}	14.78^{b}	13.33^{b}	4.032
SGR (%/d)	2.72^{a}	2.21^{b}	2.12^{b}	0.225
Survival rate, %	86.46^{a}	83.55^{b}	86.58^{a}	0.252

** Average in the same row having different superscripts (a and b) differ significantly $P \leq 0.05$*
** SED is the standard error of difference*

Table (3). Effect of partial replacement of fish meal protein by plant protein sources on feed utilization of gilthead Sea bream.

Item	Diets			SED
	33% PP	50% PP	66% PP	
Feed intake (g/fish)	7.87^{a}	5.25^{b}	4.90^{b}	1.122
FCR	3.56^{b}	3.95^{ab}	4.08^{a}	0.185
Protein utilization				
PER	0.61^{a}	0.56^{ab}	0.54^{b}	0.03
PPV (%)	6.55^{b}	8.28^{a}	6.97^{b}	0.351
Energy utilization				
EER	0.056^{a}	0.052^{ab}	0.050^{b}	0.0024
EPV (%)	8.52^{a}	7.80^{b}	7.72^{b}	0.346

** Average in the same row having different superscripts (a and b) differ significantly $P \leq 0.05$.*
** SED is the standard error of difference*

II- Effect of stocking density (regardless diets effect) on:

1- Growth performance and survival rate of gilthead Sea bream regardless of protein source.

Results of growth performance and survival rate are shown in Table (4). The results showed that insignificant differences ($P \leq 0.05$) were obtained in final weight, total weight gain, daily gain, SGR and survival rate. Growth performance parameters were highest with stocking density 55 fry/m^3 compared with stocking density 40 fry/m^3. Final body weight (g), total weight gain (g), daily gain (mg/d), SGR% and survival rates for stocking density 55 fry/m^3 were 2.03, 1.82, 20.21, 2.51 and 85.49% respectively. Growth parameters for stocking density 40 fry/m^3 were 1.53, 1.32, 14.67, 2.21 and 85.56%, respectively.

The results indicated that Stocking density of 55 fry/m^3 improved the growth performance compared with stocking density 40 fry/m^3. these results agreed with the resulted obtained by Yilmaz and Arabaci 2010 who indicated that high stocking density (44 fish/m^3) cause to improve, growth, feed efficiency and gross benefit compared to low and medium stocking densities (36 and 40 fish/m^3) in gilthead sea breams. Such trend was mentioned with growth studies reported for sea bass (Papoutsoglou *et al.*, 1998 and Sammouth *et al.*, 2009, Arctic charr (Wallace *et al.*, 1988; Jorgensen *et al.*, 1993).

Table (4). Effect of stocking density on growth performance and survival rate of gilthead Sea bream.

Item	Stocking density/m^3		SED
	40 fry/m^3	55 fry/m^3	
Initial weight (g/fish)	0.21	0.21	
Final weight (g/fish)	1.53	2.03	0.367
Total gain (g/fish)	1.32	1.82	0.367
Daily gain (mg/d)	14.67	20.22	4.139
SGR (%/d)	2.21	2.52	0.207
Survival rate, %	85.56	85.49	1.511

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

2- Feed utilization

Results of feed utilization parameters are shown in Table (5) The results showed insignificant differences (P≤0.05) were obtained in feed intake, FCR, PER, PPV, EER and EPV. Results of feed utilization parameters were highest with stocking density 55 fry/m^3 compared with stocking density 40 fry/m^3. Results showed that feed intake (g/fish), FCR, PER, PPV, EER and EPV% for stocking density 55 fry/m^3 were 6.78, 3.79, 0.0059, 7.58, 0.055 and 8.43, respectively. The same feed utilization for stocking density 40 fry/m^3 were 5.22, 3.96, 0.0057, 6.77, 0.053 and 8.2 respectively. These results indicated that the best FCR was recorded with stocking density 55 fry/m^3.

Table (5). Effect of stocking density on feed utilization of gilthead Sea bream.

Item	Stocking density/m^3		SED
	40 fry/m^3	55 fry/m^3	
Feed intake (g/fish)	5.22	6.78	0.948
FCR	3.95	3.73	0.246
Protein utilization			
PER	0.55	0.59	0.04
PPV (%)	6.77	7.58	0.636
Energy utilization			
EER	0.051	0.054	0.0036
EPV (%)	7.82	8.27	0.701

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

III- Effect of partial replacement of fish meal protein by plant protein sources and stocking density (interaction between diets and stocking density) on:

1- Growth performance and survival rate of gilthead Sea bream.

Results of growth performance parameters and survival rate of fish fed with the three different diets at both densities are shown in Table (6). Acceptance of the experimental diets was good with increased levels of fish meal.

The results cleared that the partial replacement of fish meal protein with plant protein had significant effects (P≤0.05) on the growth performance parameters (final weight, total gain, daily gain and SGR). While the highest values were obtained with the diet contained 33% PP under stocking densities 55 fry/m^3. The diets contained 50 and 66% PP under stocking densities 40 fry/m^3 had the lowest value than other treatment. Final body weight was progressively decreased with increasing PP under both densities. Final body weight, total gain, daily gain and SGR values were progressively with stocking densities 55 fry/m^3 compared with stocking densities 40 fry/m^3 under three different diets. The improvement of all growth performance parameters tested in diets containing higher levels of FM under both densities. These results led to believe that the fish meal presented in the diet contain a good smell which attract the fish to consume the diet and resulted in improving growth rate as it has better essential amino acid profile and a good source of essential minerals and vitamins.

These results agreed with the results of Gomez-Requeni *et al.* (2004); Sitja-Bobadilla *el al.* (2005) and Vega-Rubin *et al.* (2004) who obtained significant differences with growth performance parameters in Sea bream fed on plant protein sources diets compared with those fed fish meal diet. Fish fed diets high in dietary level of plant protein (SBM) generally exhibit progressive impairment of growth and increased feed conversion ratios, Such trend was mentioned by Krogdahl *et al.*, 2003 and Rumsey *et al.*, 1994. Sensitivity of gilthead sea bream to dietary level of plant protein (SBM) inclusion was higher at lower fish weights, Such trend was mentioned by Martinez-Llorens *et al.* (2007).

Growth decreased gradually with increasing dietary plant protein. This observation was in line with that of Kissil *et al.* (2000), who reported that there was an inverse relationship between growth and dietary level of plant protein, and growth was reduced in the diet for gilthead sea bream with only 30% soy bean replacement level. In contrast, most studies have shown considerable success in partial (40- 75%) or total replacement of FM with soybean, such as Atlantic halibut (Berge *et al.*, 1999), Atlantic salmon (Refstie *et al.*, 2001), common carp (Escaffre *et al.*, 1997), rainbow trout (Kaushik *et al.*, 1995; Medale *et al.*,1998; Mambrini *et al.*, 1999) and Senegalese sole (Aragao *et al.*, 2003).

Table (6). Effect of partial replacement of fish meal protein by plant protein sources and stocking density on growth performance and survival rate of gilthead Sea bream.

Diet	33% PP		50% PP		66% PP		SED
Density/m^3	40	55	40	55	40	55	
Item							
Initial weight (g/fish)	0.21	0.21	0.21	0.21	0.21	0.21	
Final weight (g/fish)	2.09^{ab}	2.74^{a}	1.29^{b}	1.79^{ab}	1.22^{b}	1.57^{ab}	0.494
Total gain (g/fish)	1.88^{ab}	2.53^{a}	1.08^{b}	1.58^{ab}	1.01^{b}	1.36^{ab}	0.494
Daily gain (mg/d)	20.8^{ab}	28.1^{a}	12.0^{b}	17.5^{ab}	11.1^{b}	15.1^{ab}	5.493
SGR (%/d)	2.55^{ab}	2.85^{a}	2.02^{b}	2.38^{ab}	1.95^{b}	2.24^{ab}	0.291
Survival rate, %	86.67	86.25	83.34	83.75	86.67	86.48	4.963

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

2- Feed utilization.

Results of feed utilization of fish fed with the three different diets at both densities are shown in Table (7). Feed intake had significantly different between treatments (P≤0.05) and value was highest with diet contained (33% PP) under stocking density 55 fry/m^3. Feed intake values were lowest with diets contained (50 and 66% PP) under stocking densities 40 fry/m^3. FCR values were not significantly different between treatments (P≤0.05), the best FCR was recorded with diet (33% PP) under stocking density 55 fry/m^3. The lowest FCR was recorded with diet (66% PP) under stocking density 40 fry/m^3. The improvements in FCR for groups fed higher levels of fish meal under both densities. PPV values had significantly different between treatments (P≤0.05) and value was relatively highest with diet (50% PP) under stocking density 55 fry/m^3. There were no significant differences in EPV values between treatments (P≤0.05) and value was relatively highest with diet (33% PP) under stocking density 55 fry/m^3. Similar results were obtained for PER and EER, although values obtained for fish fed 33% PP diet under stocking density 55 fry/m^3 were relatively highest followed stocking density 40 fry/m^3 under the same diet than those for 50 and 66% PP diets under both densities and were not significantly different at (P≤0.05). These results may due to that Sea bream could digest and assimilate the FM protein in their bodies better than plant protein.

These results agreed with the results of Gomez-Requeni *et al.* (2004); Sitja-Bobadilla *el al.* (2005) and Vega-Rubin *et al.* (2004) who obtained significant differences with feed utilization parameters in Sea bream fed on plant protein sources diet compared with those fed fish meal diet.

A reduction in diet acceptability was observed in this study when fish were fed diets with high FM replacement level by plant protein. The reduction in diet palatability usually results in a decrease in feed intake, which could in turn cause reduced growth (Kissil *et al.*, 2000; Aragao *et al.*, 2003). Diet palatability differences could have been resulted from the removal of more palatable factors (e.g. FM) replaced by plant protein (Kissil *et al.*, 2000).

The higher feed consumption observed for those fish fed higher levels of FM, Such trend was showed by Gomez-Requeni *et al.* (2004); Sitja-Bobadilla el al. (2005) and Vega-Rubin *et al.* (2004). On the other hand, the rtesult dissagreed with data obtained by Robaina *et al.* (1997) who reported higher feed consumption observed for fish fed high levels of CGM compared with those fed fish meal diet. Also Kokou *et al.* (2012) who found an increase in feed consumption with increasing bioprocessed soy product (BS) inclusion.

The higher feed intake observed by sea bream fed higher levels of FM with respect to those fed diet based on levels of plant protein sources, differs from data obtained by Robaina *et al.* (1995), who did not find any difference in feed intake of sea bream fed diets with different levels of plant protein sources througth 2 months. The lower feed intake with highest PP diet could be due to lower palatability of this feed as explained by Pereira and Oliva-Teles (2002). Similar results for PER agreed with the results of Robaina *et al.* (1997) who showed similar results for PER at feeding Sea bream fed on plant protein source diets.

Table (7). Effect of partial replacement of fish meal protein by plant protein sources and stocking density on feed utilization of gilthead Sea bream.

Diet	33% PP		50% PP		66% PP		SED
Density/m^3	40	55	40	55	40	55	
Item							
Feed intake (g/fish)	6.89^{ab}	8.84^{a}	4.50^{b}	5.99^{ab}	4.28^{b}	5.52^{ab}	1.524
FCR	3.66	3.49	4.17	3.79	4.24	4.06	0.329
Protein utilization							
PER	0.60	0.63	0.53	0.58	0.52	0.55	0.0005
PPV (%)	6.35^{b}	6.69^{ab}	7.88^{ab}	8.60^{a}	6.72^{ab}	7.02^{ab}	0.605
Energy utilization							
EER	0.055	0.057	0.049	0.054	0.048	0.050	0.0043
EPV (%)	8.28	8.67	7.39	8.11	7.46	7.75	0.620

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

IIII- Effect of partial replacement of fish meal by plant protein sources and stocking density on fish body composition of gilthead Sea bream.

Body chemical composition and energy content of gilthead Sea bream at the beginning and at the end of the experiment are shown in Table (8). The results in trial one showed that significant differences (P≤0.05) were obtained in moisture, CP, EE and GE of body composition at the end of the experimental period, however ash had insignificant differences. The lowest protein content was with diet contained (33% PP), but the highest content of EE was with diet (33% PP).

These results agreed with the results of Gomez-Requeni *et al.* (2004) who obtained significant differences with body chemical composition in Sea bream fed on plant protein sources diets compared with those fed fish meal diet, differs from data obtained by Kokou *et al.* (2012); de Francesco *et al.*

(2007); Venou *et al.* (2006); Robaina *et al.* (1997); Robaina *et al.* (1995) and Nengas *et al.* (1996) who did not find any difference in body chemical composition in sea bream fed diets with different levels of plant protein sources.

Table (8). Effect of partial replacement of fish meal protein by plant protein sources and stocking density on fish body chemical composition and energy content (% wet weight) of gilthead Sea bream.

Item	Start	Diets			SED
		33% PP	50% PP	66% PP	
Moisture (%)	71.94	74.83^{a}	72.82^{c}	73.64^{b}	0.217
CP (%)	12.83	10.86^{c}	14.60^{a}	12.86^{b}	0.087
EE (%)	7.06	9.45^{a}	7.13^{c}	8.52^{b}	0.084
Ash (%)	8.17	4.72	5.17	4.81	0.217
GE, kcal/g	1.390	1.505^{ab}	1.497^{b}	1.530^{a}	0.0104

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

Effect of concrete ponds area on:

1- Growth performance and survival rate of gilthead Sea bream.

Results of growth performance and survival rate are shown in Table (9). Acceptance of the experimental diet was good under concrete ponds area (10 m^3).

The results showed that significant differences (P≤0.05) were obtained in final body weight, total gain, daily gain and SGR, however survival rate had insignificant differences. Results of growth performance parameters were highest with concrete ponds area (10 m^3), however survival rate was relatively highest with concrete ponds area (1m^3) compared with concrete ponds area (10 m^3). Final body weight and SGR were progressively with concrete ponds area (10 m^3). Results show that final weight (g), total weight gain (g), daily gain (mg), SGR% and survival rates for concrete ponds area (10 m^3) were 8.41, 8.21, 91.06, 4.09 and 84.29%, respectively. Also growth parameters for concrete ponds area (1m^3) were 1.57, 1.36, 15.06, 2.23 and 86.48%, respectively. The results indicated that concrete ponds area (10 m^3) improved the growth performance compared with concrete ponds area (1m^3). The present results agree with data obtained by JICA 2010 who reported increased yield of sea bream. On the other hand there is a positive correlation between pond area and fish growth confirming the results previously obtained by Milstein (1995) who reported that carp and tilapia yields increased as the pond area increased and Ishak (1986) and Abouzied and Hassouna (2006) who reported that the highest growth rate of *Oreochromis niloticus* reared in hapas or net cages was recorded in fish reared in the largest hapa or cage.

2- Feed utilization.

Results of feed utilization is shown in Table (10). The results showed that significant differences (P≤0.05) were obtained in all feed utilization parameters between treatments. Feed intake and EPV values were highest with concrete ponds area (10 m^3). The best FCR was recorded with concrete ponds area (10 m^3) compared with (1m^3). PPV values were relatively highest with concrete ponds area (10 m^3). PER and EER values were similar results obtained for all treatments, although values obtained were relatively highest with concrete ponds area (10 m^3) compared with concrete ponds area (1m^3). The increase in fry final weight as the area of pond increased is desirable by fry producers. Also, FCR tended to improve as the area of pond increased but survival rate tended to decrease with 10 m^3 pond compared with the smallest area. Such results may suggest that FCR is pseudo due to the higher presence of natural feed as the pond area increased. Such increase in natural food is due to the higher fry population beside their residues (feces and feed residues). So fry tended to depend on such food beside that supplied. Also, the increase in fry raceway by increasing pond area may had a role in this connection. But the higher competition on natural and artificial food as well as the longer raceway may resulted in lower survival rate with larger pond (10 m^3). Moreover, the increase in growth rate as area of pond increased may had a role in reducing survival rate due to the expected higher predation between fries (Popma and Green, 1990).

Table (9). Effect of concrete ponds area on growth performance and survival rate of gilthead Sea bream.

Items	Concrete ponds area		SED
	1 m^3	10 m^3	
Initial weight, g	0.21	0.21	
Final weight, g	1.57^b	8.41^a	0.756
Total gain, g	1.36^b	8.20^a	0.756
Daily gain, mg	15.11^b	91.11^a	8.411
SGR, %	2.24^b	4.10^a	0.104
Survival rate, %	86.48	84.29	2.476

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

Table (10). Effect of concrete ponds area on feed utilization of gilthead Sea bream.

Item	Concrete ponds area		SED
	1 m^3	10 m^3	
Feed intake, g/fish	5.52^b	25.85^a	2.351
FCR	4.06^a	3.15^b	0.330
Protein utilization			
PER	0.54^b	0.70^a	0.00045
PPV, %	6.96^b	7.59^a	0.477
Energy utilization			
EER	0.050^b	0.064^a	0.0042
EPV, %	7.75^b	10.54^a	0.597

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

3- Body composition.

Body chemical composition and energy content of gilthead Sea bream at the beginning and at the end of experiment are shown in Table (11). The results showed that significant differences (P≤0.05) were obtained in moisture, CP, EE, ash and GE of body composition at the end of the experimental period. Moisture, EE and GE values were relatively highest with concrete ponds area (10m^3), however CP and ash values were relatively highest with concrete ponds area (1m^3).

Table (11). Effect of concrete ponds area on fish body chemical composition and energy content (% wet weight) of gilthead Sea bream.

Items	Start	Concrete ponds area		SED
		1 m^3	10 m^3	
Moisture (%)	71.94	73.64^b	74.59^a	0.299
CP (%)	12.83	12.86^a	10.94^b	0.072
EE (%)	7.06	8.52^b	10.75^a	0.166
Ash (%)	6.17	4.98^a	3.72^b	0.159
GE, kcal/g	1.390	1.530^b	1.632^a	0.019

** Average in the same row having different superscripts (a and b) differ significantly P≤0.05.*
** SED is the standard error of difference*

CONCLUSION

From the aforementioned results, it could be concluded that diets conained 33% PP and stocking density 55 fry/m^3 and pond area of 10 m^3 were the best growth performance and feed utilization under experimental conditions.

REFERENCES

Abou Zied, R.M. and M.M.E. Hassouna (2006). Evaluation of net enclosures systems on sex reversed Nile tilapia, *Oreochromis niloticus,* fry production at a commercial hatchery in Fayoum governorate. Fayoum J. Agric. Res. & Dev. 20 (1): 162-171.

Aragao, C., L.E.C. Conceiccao, J. Dias, A.C. Marques, E. Gomes and M.T. Dinis (2003). Soy protein concentrate as a protein source for Senegalese sole (*Sole senegalensis* Kaup 1858) diets: effects on growth and amino acid metabolism of post larvae. Aquacult. Res. 34, 1443- 1452.

Ashley, P.J. (2007). Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science 104: 199–235.

Barcellos, L.J., L.C. Kreutz, C. de Souza, L.B. Rodrigues, I. Fioreze, R.M. Quevedo, L. Cericato, A.B. Soso, M. Fagundes, J. Conrad, L.A. Lacerda and S. Terra (2004). Hematological changes in jundia (*Rhamdia quelen* Quoy and Gaimard *Pimelodidae*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. Aquaculture 237: 229-236.

Barton, B.A. (2002). Stress in fish: a diversity of response with particular reference to changes in circulating corticosteroids. Soc. Integ. Comp. Biol. 42, 517–525.

Berge, G.M., B. Grisdale-Helland and S.J. Helland (1999). Soy protein concentrate in diets for Atlantic halibut (*Hippoglossus hippoglossus*). Aquacult. 178, 139- 148.

De Francesco, M., G. Parisi, J. Perez-Sanchez, P. Gomez-Requeni, F. Medale, S.J. Kaushik, M. Mecatti and B.M. Poli (2007). Effect of high- level fish meal replacement by plant proteins in gilthead sea bream (*Sparus aurata*) on growth and body/fillet quality traits. Aquaculture Nutrition 13, 361-372.

Di Marco, P., A. Priori, M.G. Finoia, A. Massari, A. Mandich, and G. Marino (2008). Physiological responses of European sea bass *Dicentrarchus labrax* to different stocking densities and acute stress challenge. Aquaculture 275, 319–328.

Ellis, T., B. North, A.P. Scott, N.R. Bromage, M. Porter and D. Gadd (2002). The relationships between stocking density and welfare in farmed rainbow trout. J. Fish Biol. 61, 493–531.

Escaffre, A.M., J.L. Zambonino Infante, C.L. Cahu, M. Mambrini, P. Begot and S.J. Kaushik (1997). Nutritional value of soy protein concentrate for larvae of common carp *Cyprinus carpio* based on growth performance and digestive enzyme activities. Aquacult. 153, 63- 80.

FAO (2009). Aquaculture Production 1950-2007. FAO, Rome, Italy.

Gomez-Requeni, P., M. Mingarro, J.A. Calduch-Giner, F. Medale, S.A.M. Martin, D.F. Houlihan, S. Kaushik and J. Perez-Sanchez (2004). Protein growth performance, amino acid utilisation and somatotropic axis responsiveness to fish meal replacement by plant protein sources in gilt head sea bream (*sparus aurata*). Aquaculture 232, 493-510.

Irwin, S., J.O. Halloran and R.D. Fitzgerald (1999). Stocking density, growth and growth variation in juvenile turbot, *Scophthalmus maximus* (Rafinesque). Aquacult. 178, 77-88

Ishak, M.M. (1986). Development of fish farming in Egypt. Cage and pen (enclosure) culture. Instit. Of Oceanog. And Fish. Egypt. And IDRC Canada Report No. 4 phase II, 101 pp.

JICA (2010). Japan International Cooperation Agency. Economic performance of sea bass and sea bream culture. Mediterranean fisheries research, production and training institute. Special Publication 6.

Jorgensen, E.H., J.S. Christiansen and M. Jobling (1993). Effects of stocking density on food intake, growth performance and oxygen consumption in Arctic charr (*Sal6elinus alpinus*). Aquaculture 110, 191–204.

Kaushik, S.J., J.P. Cravedi, J.P. Lalles, J. Sumpter, B. Fauconneau and M. Laroche (1995). Partial or total replacement of fish meal by soybean protein on growth, protein utilization, potential estrogenic or antigenic effects, cholesterolemia and flesh quality in rainbow trout, (*Oncorhynchus mykiss*). Aquacult. 133, 257- 274.

Kissil, G.W., I. Lupatsch, D.A. Higgs and R.W. Hardy (2000). Dietary substitution of soy and rapeseed protein concentrates for fish meal, and their effects on growth and nutrient utilization in gilthead sea bream *(Sparus aurata L.*) Aquac. Res. 31, 595–601.

Kokou, F., G. Rigos, M. Henry, M. Kentouri and M. Alexis (2012). Growth performance, feed utilization and non-specific immune response of gilthead sea bream (*Sparus aurata L.*) fed graded levels of a bioprocessed soybean meal. Aquaculture 364-365, 74-81.

Kristiansen, T.S., A. Ferno, J.C. Holm, L. Privitera, S. Bakke and J.E. Fosseidengen (2004). Swimming behaviour as an indicator of low growth rate and impaired welfare in Atlantic halibut (*Hippoglossus hippoglossus* L.) reared at three stocking densities. Aquaculture, 230: 137-151.

Krogdahl, A., A.M. Bakke-McKellep and G. Baeverfjord (2003). Effects of graded levels of standard soybean meal on intestinal structure, mucosal enzyme activities, and pancreatic response in Atlantic salmon (*Salmo salar L*.). Aquaculture Nutrition 9, 361 –371.

Lupatsch, I., G.A. Santos, J.W. Schrama and J.A.J. Verreth (2010). Effect of stocking density and feeding level on energy expenditure and stress responsiveness in European sea bass *Dicentrarchus labrax*. Aquaculture, 298: 245–250

Mambrini, M., A.J. Roem, J.P. Cravedi, J.P. Lalles and S.J. Kaushik (1999). Effects of replacing fish meal with soy protein concentrate and of DL methionine supplementation in high-energy, extruded diets on the growth and nutrient utilization of rainbow trout, (*Oncorhynchus mykiss*). J. Anim. Sci. 77, 2990- 2999.

Martinez-Llorens, S., A.V. Monino, A. Tomas Vidal, V.J.M. Salvador, M. Pla Torres and M. Jover Cerda (2007). Soybean meal as a protein source in gilthead sea bream (*Sparus aurata L*.) diets: effects on growth and nutrient utilization. Aquaculture Research 38, 82–90.

Medale, F., T. Boujard, F. Vallee, D. Blanc, M. Mambrini, A. Roem and S. Kaushik (1998). Voluntary feed intake, nitrogen and phosphorus losses in rainbow trout, (*Oncorhynchus mykiss*) fed increasing dietary levels of soy protein concentrate. Aquacult. Living Resour. 11, 239- 246.

Merino, G.E., R.H. Piedrahita and D.E. Conklin (2007). The effect of fish stocking density on the growth of California halibut (*Paralichthys californicus*) juveniles. Aquaculture 265, 176–186.

Milstein, A. (1995). Fish management relationships in Israeli commercial fish farming. Aquaculture Int. 3(4): 292-314.

Mommsen, T.P., M.M. Vijayan, and T.W. Moon (1999). Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Rev. Fish Biol. Fish. 9, 211–268.

Montero, D., M.S. Izquierdo, L. Tort, L. Robaina and J.M. Vergara (1999). High stocking density produces crowding stress altering some physiological and biochemical parameters in gilthead sea bream, *Sparus aurata*, juveniles. Fish Physiol. Biochem. 20, 53–60.

Moyano, F. J., G. Cardenete, and M. de la Higuera (1992). Use of two vegetable by-products as protein sources in rainbow trout feeding. Anim. Prod., 55: 277-288

Nengas, I., M. Alexis and S.J. Davies (1996). Partial substitution of fish meal with soybean meal products and derivatives in diets for the gilthead sea bream *(Sparus aurata L).* Aquac. Res. 27, 147–156.

North, B.P., J.F. Turnbull, T. Ellis, M.J. Porter, H. Migaud, J. Bron and N.R. Bromage (2006). The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 255: 466-

NRC (1993). Nutrient requirements of fish. Committee on Animal Nutrition Board on Agriculture National Research Council National Academy Press, Washington DC, USA.

Papoutsoglou, S.E.G., X. Tziha and A. Vrettos (1998). Effects of stocking density on behavior and growth rate of European sea bass (*Dicentrarchus labrax*) juveniles reared in a closed circulated system. Aquacultural Engineering 18, 135–144.

Pereira, T.G. and A. Oliva-Teles (2002). Preliminary evaluation of pea seed meal in diets for gilthead sea bream (*Sparus aurata*) juveniles. Aquac. Res. 33, 1183–1189.

Popma, T.J. and B.W. Green (1990). Sex-reversal of Tilapia in earthen ponds. Int. Center Aquacult. Alabama Res. &Dev. Series No. 35, 14 p.

Refstie, S., T. Storebakken, G. Baeverfjord and A.J. Roem (2001). Longterm protein and lipid growth of Atlantic salmon (*Salmo salar*) fed diets with Partial replacement of fish meal by soy protein products at medium or high lipid level. Aquacult. 193, 91- 106.

Robaina, L., M.S. Izquierdo, F.J. Moyano, J. Socorro, J.M. Vergara, D. Montero and H. Fernandez-Palacios (1995). Soybean and lupin meals as protein sources in diets for gilthead sea bream (*Sparus aurata*): nutritional and histological implications. Aquaculture 130, 219–233.

Robaina, L., F.J. Moyano, M.S. Izquierdo, J. Socorro, J.M. Vergara and D. Montero (1997). Corn gluten and meat and bone meals as protein sources in diets for gilthead sea bream (*Sparus aurata*): Nutritional and histological implications. Aquaculture 157, 347-359.

Rumsey, G.L., A.K. Siwicki, D.P. Anderson and P.R. Bowser (1994). Effect of soybean protein on serological response, non-specific defense mechanisms, growth, and protein utilization in rainbow trout. Veterinary Immunology and Immunopathology 41, 323–339.

Sadek, S. (2000). Sea bream culture in Egypt; status, constraints and potential. Fish Physiology and Biochemistry, , Volume 22, Number 2, Pages 171-178.

Sammouth, S., E.R. d'Orbcastel, E. Gasset, G. Lemarie, G. Breuil, G. Marino, J. Coeurdacier, S. Fivelstad and J. Blancheton (2008). The effect of density on sea bass (Dicentrarchus labrax) performance in a tank-based recirculating system. Aquacult. Eng. 40, 72–78.

Santigosa, E., I. Garcia-Meilan, J.M. Valentin, J. Perez-Sanchez, F. Medale, S. Kaushik and M.A. Gallardo (2011). Modifications of intestinal nutrient absorption in response to dietary fish meal replacement by plant protein sources in sea bream (*Sparus aurata*) and rainbow trout (*Onchorynchus mykiss*). Aquaculture 317, 146-154.

Schram, E., J.W. Van der Heul, A. Kamstra and M.C.J. Verdegem (2006). Stocking density-dependent growth of Dover sole (*Solea solea*). Aquaculture, 252: 339-347.

Sitja-Bobadilla, A., S. Pena-Llopis, P. Gomez-Requeni, F. Medale, S. Kaushik and J. Perez-Sanchez (2005). Effect of fish meal replacement by plant protein sources on non-specific defence mechanisms and oxidative stress in gilthead sea bream (*Sparus aurata*). Aquaculture 249, 387-400

SPSS. (2010). Statistical Package For Social Science (for Windows). Release 16 Copyright (C), SPSS Inc., Chicago, USA.

Tacon, A.G.J. and Jackson, A.J. (1985). Utilization of conventional and unconventional protein sources in practical fish feed. A review. in: Cowey, C.B., Mackie, A.M., Bell, J.G. (Eds.), Nutrition and Feeding in Fish. Academic Press, London, pp. 118-145.

Turnbull, J., A. Bell, C. Adams, J. Bron and F. Huntingford (2005). Stocking density and welfare of cage farmed Atlantic salmon: application of a multivariate analysis. Aquaculture, 243: 121-132.

Vega-Rubin, S.C., P. Rojas, P. Gomez-Requeni, A. Albalat, J. Gutierrez, F. Medale, S.J. Kaushik, I. Navarro and J. Perez-Sanchez (2004). Nutritional assessment of somatolactin function in gilthead sea bream (*Sparus aurata*): concurrent changes in somatotropic axis and pancreatic hormones. Comparative Biochemistry and Physiology, Part A 138, 533-542.

Venou, B., M.N. Alexis, E. Fountoulaki and J. Haralabous (2006). Effects of extrusion and inclusion level of soybean meal on diet digestibility, performance and nutrient utilization of gilthead sea bream (*Sparus aurata*). Aquaculture 261, 343–356.

Vijayan, M.M., J.S Ballantyne, and J.F. Leatherland (1990). High stocking density alters the energy metabolism of brook charr, *Salvelinus fontinalis*. Aquaculture 88, 371–381.

Wallace, J.C., A. Kolbeinshavn and T.G. Reisnes (1988). The effects of stocking density on early growth in Arctic charr, *Salvelinus alpinus* (L.). Aquaculture 73, 101–110.

Wedemeyer, G.A. (1997). Effects of rearing conditions on the health and physiological quality of fish in intensive culture. In fish stress and health in aquaculture, Iwama, G.K., A.D. Pickering, J.P. Sumpter abd S.B. Schreck (Eds.). 1st Edn, Cambridge University Press, Cambridge, ISBN-10: 0521555183, pp: 35-71.

Yeroulanos, L.B. (2010). General fisheries commission for the Mediterranean. Studies and reviews, Synthesis of Mediterranean marine finfish aquaculture a marketing and promotion strategy. Food and Agriculture Organization of the United Nations. No. 88.

Yilmaz. Y. and M. Arabaci (2010). The influnece of stocking density on growth and feed efficiency in gilthead sea bream *Sparus aurata.* J. Anim. Vet. Advances 9 (8): 1280-1284.

تأثير استبدال بروتين مسحوق السمك بمصادر بروتين نباتى وكثافة التخزين ومساحة الاحواض الاسمنتية على انتاج زريعة الدنيس.

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أجريت تجربتين علي زريعة أسماك الدنيس. التجربة الأولي لدراسة تأثير الاستبدال الجزئي لبروتين مسحوق السمك بالبروتين النباتي (٣٣%، ٥٠% و ٦٦%) تحت تأثير كثافتين تخزين (٤٠، ٥٥ زريعة/م٣). الثانية درست تأثير حجم الحوض (١، ١٠ م٣). وقد اظهرت النتائج في التجربة الاولى أن الاستبدال الجزئي لبروتين مسحوق السمك بالبروتين النباتي كان لة تأثير معنوي علي مظاهر النمو متمثلة في الوزن النهائي والزيادة الكلية ومعدل النمو النوعي وكان أعلي قيم تم الحصول عليها مع العليقة التي أحتوت علي ٣٣% بروتين نباتي. وتراوح معدل البقاء والذى اختلف معنويا يبين ٨٣.٥٥% - ٨٦.٥٨% ولوحظ ارتفاع معدل استهلاك الغذاء للعلائق المحتوية علي نسبة كبيرة من بروتين مسحوق السمك (٣٣% بروتين نباتى) مقارنة بالعلائق الاخرى تحت معدل كثافة ٥٥ سمكة/ م٣. وبالنظر لتأثير الكثافة اوضحت النتائج أن ٥٥ زريعة/م٣ حسنت مظاهر النمو ومعدل التحويل الغذائى مقارنة بالكثافة ٤٠ زريعة/م٣ وكذلك أثرت العلائق على التركيب الكيماوى للأسماك معنويا وكانت عليقة ٥٠% بروتين نباتى اكثر تأثيرا على بروتين الجسم والاقل على مستخلص الايثير.

اظهرت نتائج التجربة الثانية ان مساحة الحوض كان لها تأثير معنوي علي مظاهر النمو و تحسن معدل التحويل الغذائي وقد سجل الحوض الكبير (١٠ م٣) اعلى القيم .