

IMPACTS OF USING YEAST, IODINE AND THYROXIN IN SEA BREAM (*SPARUS AURATA*) DIETS ON FISH PERFORMANCE

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SUMMARY

This study was conducted to investigate the effect of adding either brewer yeast as a probiotic, iodine or thyroxin with yeast as synbiotics to sea bream (*Sparus aurata*) fingerlings diets on; growth performance, feed utilization, body composition, biometric measurements indices and some serum constituents of sea bream. Acclimatized fish were randomly distributed in 12 glass aquaria and stocking density was 15 fish per aquarium with an average weight 1.9 ± 0.1 g/fish as triplicate groups per treatment. The 1st group was considered as a control which fed on the basal diet (C). Three tested diets are formulated by adding 2g yeast/kg diet (Y), 2g yeast+0.01g potassium iodide/kg diet (YI) and 2g yeast+0.05 g thyroxin /kg diet (YT4) respectively for second, third and fourth groups. In the present work, addition of yeast with thyroxin followed by yeast alone to sea bream (*Sparus aurata*) fingerlings diets significantly improve growth performance, feed utilization efficiency and the fish biochemical composition comparing with the control one. The lowest growth performance and feed utilization values were recorded in fish fed diet supplemented with iodine when compared with corresponding values in fish of control group. Results of biometric measurements showed that adding iodine to fish diet significantly reduced gut length. Adding either iodine or thyroxin significantly increased serum lipase and decreased amylase contents.

Keywords: *Sparus aurata, yeast, Iodine, thyroxin, synbiotic*

INTRODUCTION

The demand of animal protein for human consumption is currently on the rise and is largely supplied with terrestrial farm animals. Aquaculture, however, is an increasingly important option in animal protein production. This activity requires high-quality feeds with high protein content, which should contain not only necessary nutrients but also complementary additives to keep organisms healthy and for better growth. Some of the most utilized growth-promoting additives are hormones, antibiotics, ionophores and some salts (Klaenhammer and Kullen, 1999). Though these do promote growth, their improper use can result in adverse effects on the animal and the final consumer, as well as lead to increase resistance against pathogenic bacteria in the case of antibiotics. In recent years, the concept of "functional foods" has developed in human and animal nutrition. A "functional nutrient" can be further defined as a dietary ingredient that exerts possible positive effects on health in addition to its direct role as a nutrient (Rasha *et al.*, 2010). Dietary "probiotics" such as yeast are included under this category. Probiotic is defined, in the strict sense, as "a viable microbial dietary supplement that beneficially affects the host through its effects in the intestinal tract" (Roberfroid, 2000). It can improve the nutrition level of aquacultural animals and improve immunity of cultured animals to pathogenic microorganisms (Ringø *et al.*, 2012).

Thyroid hormones are essential for vertebrate development. Triiodothyronine (T3) is the major hormone secreted from thyroid gland and is supposed to be the active precursor for thyroxin (T4). These hormones regulate the level of metabolic activity in fish and have wide influence on cellular oxidation,

neuromuscular control, circulatory dynamics, nutrient metabolism and growth (Johannes *et al.*, 2012; Cray *et al.*, 2013). The metamorphic climax is induced by a surge of thyroid hormones, and they may also regulate development before and after the metamorphosis process (Hulbert, 2000). In zebra fish (*Danio rerio*) thyroid hormones play a role in regulating the differentiation of the pectoral fins and determining the transition from the larval stage to the juvenile stage (Brown, 1997). Garg (2007) studied the effects of oral administration of l-thyroxin (T₄) on growth performance, body composition, and some aspects of nutritional physiology in (*Channa punctatus* and *Heteropneustes fossilis*). He found that irrespective of the species, fish fed diets containing lower doses of T₄ (up to 50 mg/ kg of diet in *C. punctatus* and up to 100 mg/ kg of diet in *H. fossilis*) showed significantly ($P < 0.05$) higher growth (in terms of live weight and length gain, specific growth rate, percentage gain in body weight and condition factor), low feed conversion ratio, high nutrient retention, high apparent protein digestibility, and high digestive enzyme activity. Viscero-somatic (VSI) and hepato-somatic (HSI) values were also high in fish fed on low dietary T₄ levels. Woo *et al.* (1991) showed that adding of 3, 5, 3'-triiodo-thyronine (T₃) in the diet of under yearling red sea bream (*Chrysophrys major*) increased growth rate, appetite, food conversion efficiency and activities of intestinal enzymes. There were no changes in the muscle content of water, protein, lipid and glycogen. Serum concentrations of total protein, albumin, globulin, α -amino acids, glucose, ammonia and calcium were increased by the treatment whereas the serum concentrations of free fatty acids, cholesterol and triglyceride remained unaltered. The results suggested that in the red sea bream thyroxin stimulated protein and carbohydrate but not lipid metabolism and that the hormone promoted growth by improving appetite, digestion and absorption.

The role of trace elements in biological systems has been described in several animals; however, the knowledge in fish is mainly limited. Investigations in fish are comparatively complicated as both dietary intake and waterborne mineral uptake have to be considered in determining the mineral budgets. The importance of trace minerals as essential ingredients in diets, although in small quantities, is also evident in fish. Iodine (I) is essential for vertebrates where it is utilized by the thyroid follicles to produce I containing thyroid hormones, thyroxin (T₄) and triiodothyronine (T₃) (Akchurina, 2013; Vilutis, 2014) which regulate the level of metabolic activity, body growth and temperature maintenance (Zhao *et al.*, 2014). The dietary iodine is easily absorbed in the digestive tract (Gregory and Eales, 1975). Although the iodine requirement of most fish has not been established, Lovell (1979) has recommended a minimum dietary level of 2.8 mg I / kg diet. Age, physiological state and stress factors considerably influence the requirement for this mineral (Watanabe *et al.*, 1997).

Iodine deficiency can lead to thyroid enlargement, termed goitre (Maier *et al.*, 2007) and alter circulating thyroid hormone levels and ratios (Ruz *et al.*, 1999). As sea water contains more iodine than freshwater so iodine deficiency signs appear more in fresh water fish (Gregory and Eales, 1975). Commonly, artemia and other prey species used as food for cultured marine larvae, are enriched by adding water-soluble micronutrients, such as potassium iodide (KI), directly to the enrichment water to facilitate uptake via drinking or adsorption (Moren *et al.*, 2006; Hamre *et al.*, 2008)

The present work was conducted to investigate the effects of dietary addition of yeast (*Saccharomyces cerevisiae*) as a probiotic, yeast with iodine or yeast with thyroxin as synbiotic growth enhancers, on growth, feed utilization efficiency, survival rates, biochemical composition, biometric measurements indices and also some serum constituents of sea bream *Sparus aurata* fingerlings.

MATERIALS AND METHODS

Experimental fish facilities and feeding regime:

Gilthead sea bream fingerlings were obtained from El-Wafa Marine Fish Hatchery (Ismailia governorate) and transferred to Fish Nutrition Lab. (National institute of Oceanography and Fisheries, NIOF), Alexandria, Egypt. Fish were acclimatized to the laboratory conditions for 20 days. Healthy fish were randomly distributed in 12 glass aquaria (100 L each) at a density of 15 fish per aquarium with an average weight 1.9 ± 0.1 g/fish as triplicate groups per treatment. The aquaria were daily cleaned and excreta were siphoned before the first feeding. The experiment was under the natural photoperiod (approximately 12/12 h light/dark). Water quality parameters were monitored weekly throughout the experimental period using standard APHA, 1995 methodology. The average values of these parameters were: $20 \pm 2^\circ\text{C}$ water temperature, 37.5 ± 0.5 ppm salinity, 7 ± 0.2 mg/l dissolved oxygen and 7.9 ± 0.1 PH. Fish were fed the test diets until visual apparent satiation, 7 days a week for 84 days. Fish in each aquarium were counted and weighed (collectively) biweekly throughout the feeding trials.

Experimental diets:

Isonitrogenous (~45% crude protein), isocaloric (~477 Kcal/100g gross energy, GE) experimental diets were prepared as shown in Table (1). The ingredients were blended with additional water to make a paste of each diet. The pastes were pelleted into the appropriate size suitable for the experimental fish size. The diets were dried into an oven thermostatically regulated at 60°C for 24 hours and stored in plastic bags at -20°C until use.

Table (1): The composition and chemical analysis (% on dry matter basis) of the experimental diets.

Ingredients	Experimental diets composition/kg.			
	Control (C)	Yeast (Y)	Yeast + Iodine (YI)	Yeast+Thyroxine (YT4)
Fish meal (68% CP)	500	500	500	500
Soybean meal	220	220	220	220
Yellow corn	60	60	60	60
Wheat bran	80	80	80	80
Wheat flour	59.5	57.5	57.49	57.45
Premix ¹	20	20	20	20
Fish oil	30	30	30	30
Sunflower oil	30	30	30	30
Dicalcium phosphate	0.5	0.5	0.5	0.5
Yeast (Y) ²	-	2	2	2
potassium iodide (I) ³	-	-	0.01	-
Thyroxin (T4) ⁴	-	-	-	0.05
	1000	1000	1000	1000
	Chemical composition %			
Dry matter (DM)	96.7	97.5	97.5	97.5
Crud protein (CP)	44.6	43.9	44.3	44.8
Ether extract	11.3	11.1	11.5	10.9
Crude fiber	2.9	2.8	2.9	2.8
Nitrogen free extract (NFE) ⁵	28.8	29.9	29.2	29.2
Ash	12.4	12.3	12.1	12.3
Gross energy (kcal/100g DM) ⁶	476.4	475.2	478.6	475.4
P:E ratio (mg CP: kcal GE)	93.6	92.4	92.6	94.2

¹Vit./min. Premix (mg kg⁻¹); ¹Premix Composition: - Each 1 kg contains Vit A (400000 i.u.), Vit D3 (100000 i.u.), Vit E (230 mg) Vit K3 (165mg) Vit B1 (300 mg), Vit B2 (80 mg), Vit B6 (200 mg), Vit B12 (1mg), Vit C (650 mg), Niacin (1000 mg), Methionine(3000 mg), Choline chloride (10000 mg), Folic acid (100 mg), Biotin (2 mg), Pantothenic acid (220 mg), Magnesium sulphate (1000 mg), Copper sulphate (1000 mg), Iron sulphate (330mg), Zinc sulphate (600 mg), Cobalt sulphate (100 mg), Calcium carbonate up to (1000 mg).

²(*Saccharomyces cerevisiae*)

³(I) Potassium Iodide, El- Nasr Pharmaceutical Chemicals Co. Dose according to Love11 (1979)

⁴(T4) Eltroxin[®] tablets 100µg Glaxo Smith Kline Co. (50 mg/kg diet). Dose according to Garg (2007)

⁵NFE = 100 - [% Ash + % lipid + % protein + % Fiber]

⁶Gross energy (GE) was calculated as 5.64, 9.44 and 4.11 kcal/100g for protein, lipid and NFE, respectively (NRC, 1993).

Data collection and samples analyses:

At the end of the experiment, fish of each aquarium were weighed collectively and average final weight (g/fish) was calculated. Total fish lengths (cm), total body weights (g) were measured for each aquarium. Blood samples from 6 fish for each aquarium were collected by suction from the caudal peduncle and transferred to centrifuge tubes and allowed to clot at room temperature. Serum was then separated by centrifugation at 3000 (rpm) for 10 minutes and stored at -20°C until further analysis. Serum total protein (g/dl) was determined calorimetrically using kits supplied by El-Nasr Pharmaceutical Chemicals Co. (Egypt). Serum total lipid (g/dl); lipase and amylase activities were determined calorimetrically using commercial kit's Diamond[™] Diagnostic Co. (Egypt). Serum thyroxin (T4) (µg/dl) concentrations were determined by ELISA technique using kits of DIMAGes. F. Diagnostika mbH, Germany.

Chemical analysis of diets and fish:

After blood samples collection, the abdominal cavity of fish from each aquarium was opened to remove viscera. Gut length (cm), liver and viscera weights (g) were recorded. Pooled samples of the rest of fish for each aquarium were sacrificed and frozen at -20°C for subsequent body composition analysis. The tested diets and whole-fish body from each treatment were analyzed according to the standard methods of AOAC (1995) for moisture, crude protein, crude fat (ether extract) and ash.

Fish performance indices:

Growth rate and feed utilization efficiency indices have been obtained as mentioned by Ballestrazzi *et al.* (1994) as follows:

$$WG = FW - IW \text{ (g / fish)}$$

$$PWG = 100 \times [(\text{final fish weight (g)} - \text{initial fish weight (g)}) / \text{initial fish weight}]$$

$$SGR = 100 \times [(\ln \text{ final fish weight}) - (\ln \text{ initial fish weight})] / \text{experimental days}$$

$$FCR = \text{feed fed (g) (dry weight)/weight gain (g)}$$

$$PER = \text{weight gain (g) / protein fed (g)}$$

$$PPV = 100 [\text{protein gain (g) / protein fed (g)}]$$

$$\text{Energy utilization (\%)} = 100 [\text{Energy gain (kcal/100g) / Energy intake}]$$

Biometric indices:

Some biometric indices as hepatosomatic (HSI), viscerosomatic (VSI) indices, condition factor (CF) and relative gut length (RGL) were calculated. HSI was determined according to Schreck and Moyle (1990) as follows: $HSI = 100 \times [\text{liver weight (g)/total body weight (g)}]$. VSI was estimated according to Ricker (1979) as follows: $VSI = 100 \times [\text{viscera weight (g)/total body weight (g)}]$. $CF = 100 \times (TW/L^3)$ where TW= Total weight (g); L= Total fish length (cm). Relative Gut length (RGL) was determined according to Al-Hussini (1947) as follows: $RGL = \text{absolute gut length (cm)/ TL (cm)}$.

Statistical analyses:

Mean value and standard error (mean \pm SE) for each parameter of all treatments was first calculated. The results were subjected to one way analysis of variance (ANOVA) to test the effect of treatment inclusion on fish performance. Data were analyzed using SPSS program (SPSS, 1997), Version 16. Differences between means were compared using Duncan multiple range test at $P < 0.05$ level.

RESULTS AND DISCUSSION

Growth performance and feed utilization efficiency:

The average initial weight (IW), final weight (FW), total weight gain (TWG), specific growth rate (SGR) and survival rate (SR %) of sea bream fingerlings are shown in Table (2). At the end of the experiment, results showed that all growth performance parameters of fish fed diets containing yeast with or without thyroxin were significantly ($P < 0.05$) higher than those fed either C or YI diet. Survival rates showed insignificant changes among groups ($P > 0.05$). Addition of potassium iodide to fish diet significantly decreased the growth performance parameters comparing with fish fed either Y or YT4 diets but results were insignificantly lower comparing to C group. Table (2) also showed the results of feed utilization efficiency of sea bream at the end of the feeding trial and they supported the results of growth performance parameters. Least FI and worst FCR values were recorded in YI group. Otherwise, best feed conversion ratio (FCR) was obtained for fish fed YT4 diet, followed by fish fed Y diet. Protein efficiency ratio (PER) and protein productive value (PPV) of the experimental fish were improved as a result of addition of thyroxin in YT4 group noticing that, protein productive value (PPV) is significantly the highest ($P < 0.05$) when compared with all other groups. Furthermore, energy utilization (EU, %) values in YI group was significantly lower ($P < 0.05$) when compared with Y and YT4.

In the present work, addition of either yeast alone or yeast with thyroxin to sea bream (*Sparus aurata*) fingerlings diets significantly improve growth performance, feed utilization efficiency and the fish biochemical composition comparing with the control one. The present results obtained after administration of yeast in sea bream diets agree with previous works on other fish species as rohu (*Labeo rohita*), catla (*Catla catla*), mrigal (*Cirrhinus mrigala*), pollack (*Pollachius pollachius*), Nile tilapia (*Oreochromis niloticus*), hybrid striped bass (*Morone chrysops* \times *M. saxatilis*), carp (*Cyprinus carpio*) (Mohanty *et al.*, 1993, 1996; Swain *et al.*, 1996; Gatesoupe 2002, 2007; Lara-Flores *et al.*, 2003; Li and Gatlin, 2005; Noh *et al.*, 1994 respectively) where enhanced growth was associated with dietary supplementation of yeast. Yeast and yeast-based products that used as a supplement in diets of various

fish species may serve as an excellent health promoter and thus improve growth, feed intake and disease resistance of cultured fish (Li and Gatlin, 2005). Abdel-Tawwab *et al.* (2006) reported that better feed intake was recorded in fish fed yeast supplemented diets (1–5 g/kg diet) and they also illustrated that enhancement in fish appetite led to better feed utilization and consequently enhanced fish growth. The positive effects of using yeast in fish diets may attributed to that they are rich source of protein and B-complex vitamins so can be used successfully as a complementary protein source in fish diet (Tacon, 1994).

Table (2): Growth performance and feed utilization efficiency of sea bream fingerlings after addition of potassium iodide and thyroxin to diet containing yeast (mean ± SE).

Item	Experimental diets			
	Control (C)	Yeast (Y)	yeast + iodine (YI)	yeast + thyroxin (YT4)
Initial body weight (IW, g/fish)	1.88±0.01	1.89±0.01	1.89±0.01	1.9±0.01
Final body weight (FW, g/fish)	7.41±0.05 ^b	8.5±0.038 ^a	6.9±0.23 ^b	8.9 ±0.34 ^a
Total weight gain (TWG, g/fish)	5.53±0.05 ^b	6.61±0.38 ^a	5.02±0.22 ^b	7.08±0.34 ^a
Specific growth rate (SGR, %/d)	2.04±0.01 ^b	2.24±0.07 ^a	1.91±0.05 ^b	2.30±0.05 ^a
Feed intake (FI, g/fish)	11.79±0.29 ^{ab}	12.62±0.12 ^a	10.99±0.16 ^b	12.12±0.42 ^a
Protein intake (PI, g/fish)	5.08±0.13 ^{ab}	5.49±0.05 ^a	4.77 ±0.07 ^b	5.27±0.18 ^a
Feed conversion ratio (FCR)	2.13±0.066 ^a	1.92±0.12 ^{ab}	2.19±0.10 ^a	1.71±0.09 ^b
Protein efficiency ratio (PER)	1.09±0.03 ^b	1.20±0.07 ^{ab}	1.05±0.10 ^b	1.35±0.07 ^a
Protein productive value (PPV %)	20.25±0.63 ^c	24.49±1.49 ^b	20.59±0.99 ^{bc}	28.51±1.48 ^a
Energy utilization (EU, %)	11.16±0.33 ^{ab}	13.02±0.71 ^a	9.48±0.41 ^b	12.84±0.63 ^a
Survival rate (SR, %)	98±2.08	100±0	100±0	99±2.45

Means in the same rows having different letters are significantly ($P < 0.05$) different.

Also, *Saccharomyces cerevisiae*, contains immune stimulating compounds such as β-glucan, nucleic acids as well as mannan oligosaccharides and accordingly has the capability to enhance immune responses (Gatesoupe, 2007). Also, Peulen *et al.* (2002) reported that the effect of yeast may be attributed to its ability to release spermine and spermidine in the digestive tract, which play a fundamental role in proliferating fast growing and regenerating tissues. This conclusion is supported by Péres *et al.* (1997) who reported an increase in the survival of sea bass larvae fed diets containing purified spermine. In addition, Waché *et al.* (2006) suggested that yeasts are promising candidates as probiotics, because of their abilities to produce polyamines and to adhere and grow in the intestinal mucus of fish and reported that the maturation enhancement in the digestive tract of marine fish larvae was due to high secretion of spermine and spermidine by the yeast. The valuable constituents of yeasts may explain the present results where fish fed Y diet shows an improvement in feed utilization indices and the highest serum total protein among all groups. Many other authors attributed feed utilization enhancement by the ability of yeast to play a role in enhancing feed intake, net protein utilization, energy retention and the high nutrient digestibility (Abdel-Tawwab *et al.*, 2008) or changes in their synthesis and/or deposition rate in muscle (Soivio *et al.*, 1989). The present study showed that sea bream fed yeast with iodine (YI) had lower results in growth performance, feed utilization efficiency. These results may be explained by the ability of sea bream, as a marine fish, to absorb iodine for osmoregulation from the surrounding water via their gills and from the diet where it is easily absorbed in the digestive tract (Gregory and Eales, 1975) and consequently the level of iodine in fish of YI group become higher than normal. Many literatures revealed that, decreased growth, mental retardation, reduced egg hatchability, increased mortality and decreased fertility have been observed in terrestrial vertebrates fed insufficient (Robertson *et al.*, 2008; Dong *et al.*, 2011) or excessive iodine (Baker *et al.*, 2003; Baker, 2004). In contrast, Penglase *et al.* (2013) recorded an increase in Atlantic cod (*Gadus morhua*) larval iodine concentrations by 3 and 7 fold compared to control during the rotifer feeding period and they found no differences in growth were observed, They suggested that iodine toxicity in fish larvae may be determined to a greater extent by iodine bioavailability and nutrient interactions rather than by body burdens of iodine. Also, the present results disagree with some other literatures as Ribeiro *et al.* (2011 & 2012) on Senegalese sole (*Solea senegalensis*) and Witt *et al.* (2009) on Pacific thread fin (*Polydactylus sexfilis*) larvae which fed on iodine enriched rotifers and artemia and they found that this enrichment prevented the development of goitre and increased growth and survival. They reported that, iodine supplementation may improve growth and development in marine fish larvae because their requirements may mirror the higher levels of iodine found in their natural feed. Contradictory between results may be explained by the difference in fish species and difference in age between larval and fingerlings fish in the ability of absorption of iodine from ambient water and diet.

Body composition:

Chemical composition of initial sea bream and that fed the experimental diets at the end of feeding trial are showed in Table (3). Addition of thyroxin in YT4 diet resulted in a significant increase in protein deposition comparing with those fed other diets. Total lipid contents were reduced significantly ($p < 0.05$) after addition of either iodine or thyroxin in the fish diet relative to fish fed either control or yeast diet. Values of ash contents showed significant variations among groups.

Table (3): Body composition of sea bream fed Y, YI and YT4 diets for 84 days (mean \pm SE).

Item	Initial fish	Experimental diets			
		Control (C)	Yeast (Y)	Yeast + Iodine (YI)	Yeast + Thyroxin (YT4)
Moisture	73.38 \pm 1.01	68.8 \pm 0.52 ^a	66.7 \pm 0.39 ^b	67.6 \pm 0.50 ^{ab}	66.7 \pm 0.29 ^b
Crude protein	67.47 \pm 1.59	58.3 \pm 0.44 ^b	59.3 \pm 0.55 ^b	59.1 \pm 0.54 ^b	61.4 \pm 0.40 ^a
Lipid	11.76 \pm 1.59	26.0 \pm 0.26 ^a	26.1 \pm 0.24 ^a	21.1 \pm 0.28 ^c	23.2 \pm 0.27 ^b
Ash	20.35 \pm 1.59	15.3 \pm 0.06 ^b	14.5 \pm 0.10 ^d	18.9 \pm 0.15 ^a	14.9 \pm 0.10 ^c

Means in the same rows having different letters are significantly ($P < 0.05$) different.

Abdel-Hamid *et al.* (2014) reported that addition of 1.5 g/kg Bio-yeast to the African catfish diet led to obtain fish with high protein and low fat contents; hence, led to improvement in cultured fish performance and quality, however, the present results of fish chemical composition indicates no significant differences in protein and lipid contents between fish fed either control or yeast diet and this contradictory in results may attributed to differences in fish species and consequently fish physiology.

Supplement of thyroxin with yeast in sea bream diet led to superiority of results comparing with that recorded when yeast was only used in fish diet. This may be explained by that thyroxin, as a thyroid hormone, is an essential hormone for vertebrate development (Johannes *et al.*, 2012; Cray *et al.*, 2013). Power *et al.* (2001) suggested that most vertebrates are unable to grow and reach their normal adult form without thyroid hormones. Thyroid hormones are also essential for the normal function of several physiological systems in fish, including cardiovascular, skeletomuscular and digestive systems). The processes and pathways mediating the intermediary metabolism of carbohydrates, lipids and proteins are all affected by thyroid hormones (THs) in almost all tissues (Moreno *et al.*, 2008) and most of these compounds have interesting properties: counteracting lipid accumulation, reducing cholesterol level and increasing lipid metabolism without cardio toxic effects and this conclusion may explain the significant decrease in lipid contents and increase in serum lipase concentrations in fish fed either YI or YT4 diets comparing with fish fed C and Y diets. The modification of thyroid hormone metabolism during ontogenesis is one of the reasons of changes in lipid composition and function of cell nuclei and its other structures (Nikitin and Babenko, 1989), so atherosclerosis and obesity may be a result of the thyroid dysfunction and modulation of the cellular lipid metabolism. Thyroid hormones influence gene expression in virtually all tissues and hence play important roles in mediating cellular metabolism and normal development (Soldin *et al.*, 2008). The present results show significant increase in fish protein deposition after administration of thyroxin in fish diet. These results are parallel with those of Nagaraju and Sunitha (2013) who mentioned that total protein and RNA content of skeletal muscle were increased significantly in the fish after T4 injection. They referred the significant protein deposition in skeletal muscle to the anabolic effect of the thyroid hormones and their actions were concentrated on transcription and translation systems involving mRNA and ribosome, the protein synthesizing units of the cells. These results may interpret the difference between impacts of addition of yeast only that increase serum total protein and the effect of yeast with thyroxin that led to reduction of protein in serum to near the control level.

Biometric measurements and serum constituents:

At the end of feeding trial hepatosomatic (HSI), viscerosomatic (VSI) indices as well as condition factor (CF) and relative gut length (RGL) of experimental fish groups were calculated and summarized in Table (4). Value of HSI in fish group fed YI diet was significantly the highest relative to all other fish groups followed by fish fed YT4 comparing with two other fish groups, meanwhile, the highest VSI value was recorded in YT4 fish group. The present results are parallel to those of Garg (2007) who concluded that adding T4 in *Channa punctatus* and *Heteropneustes fossilis* diets increase HSI and VSI values. On the other hand, CF and RGL are significantly decreased in fish fed YI diet, compared to other groups. The present data also indicate significant reduction in relative gut length in fish fed YI diet by about 26 %

comparing with control and other fish groups. These results are agree with those of Carver and Frieden (1977) who reported that during spontaneous metamorphosis of bullfrog tadpoles; gut length/body length decreased by stage XVIII and reached maximum reductions of 85 % by stage XXII when triiodothyronine was injected intraperitoneal at stages X–XII.

Table (4): Biometric measurements (mean ± SE) of sea bream fed Y, YI and YT4 diets for 84 days.

Item	Experimental diets			
	Control (C)	Yeast (Y)	Yeast + Iodine (YI)	Yeast + Thyroxin (YT4)
Hepatosomatic indices	1.36±0.16 ^c	1.38±0.23 ^c	1.70±0.32 ^a	1.42±0.17 ^b
Viscerosomatic indices	4.68±0.66 ^b	4.60±0.30 ^b	4.68±0.29 ^b	5.17±0.14 ^a
Condition factor	1.51±0.04 ^a	1.46±0.03 ^a	1.27±0.04 ^b	1.45±0.05 ^a
Relative gut length	1.07±0.08 ^a	1.07±0.08 ^a	0.79±0.015 ^b	1.09±0.04 ^a

Means in the same rows having different letters are significantly ($P<0.05$) different.

Serum constituents of experimental fish are shown in Table (5) and the highest serum total protein, total lipid and amylase concentrations were recorded in fish fed Y diet (3.3, 1.3 g/dl and 1.2 u/l, respectively). Osman *et al.* (2010) recorded an increase in serum total protein when fed Beaker's yeast, *Saccharomyces cerevisiae* to *Oreochromis niloticus*. Also, Abdel-Tawwab *et al.* (2008) mentioned that yeast supplementation in fry Nile tilapia increased serum glucose, lipid, protein, albumin, and globulin values up to 1.0 g yeast/kg diet after which those parameters decreased. The concentration of thyroid hormone (T4) was significantly ($P<0.05$) the lowest in YT4 group comparing with all other fish groups. Lipase contents in YI and YT4 had the same value and significantly higher than that in Y or C group. Addition of thyroxin to sea bream diet decreases serum lipid content which appears logically with the increase in serum lipase enzyme. The same results were reported by Sheridan (1986) who concluded that, T4 stimulates lipid mobilization from mesenteric fat and enhance lipolysis which was indicated by decreasing in total lipids, primarily as triacylglycerols, and increasing lipolytic enzyme (triacylglycerol lipase) activity in the liver and dark muscle of coho salmon, *Oncorhynchus kisutch*. The significant reduction in serum thyroxin level in YT4 group may be due to negative feedback loop because when the levels of the thyroid hormones, thyroxin and triiodothyronine increase, they prevent the release of both thyrotropin-releasing hormone and thyroid stimulating hormone (Johannes *et al.*, 2012). This system allows the body to maintain a constant level of thyroid hormones in the body. The present results suggest that, in the gilthead sea bream, iodine and thyroxin stimulated lipid but not carbohydrate metabolism.

Table (5) Serum constituents (mean ± SE) of sea bream fed Y, YI and YT4 diets for 84 days.

Item	Experimental diets			
	Control (C)	Yeast (Y)	Yeast + Iodine (YI)	Yeast + Thyroxin (YT4)
Total protein g/dl	2.5±0.06 ^b	3.3±0.05 ^a	2.5±0.04 ^b	2.7±0.04 ^b
Total Lipid g/dl	1.1±0.06 ^b	1.3±0.14 ^a	1.17±0.13 ^{ab}	1.0±0.19 ^b
(T4) µg/dl	4.1±0.16 ^a	3.8±0.07 ^a	4.1±0.04 ^a	3.0±0.10 ^b
Lipase u/l	7.5±0.50 ^c	11.3±0.85 ^b	15.0±1.20 ^a	15.0±0.04 ^a
Amylase u/l	1.0±0.1 ^{ab}	1.2±0.1 ^a	0.7±0.1 ^b	0.4±0.0 ^c

Means in the same rows having different letters are significantly ($P<0.05$) different.

CONCLUSION

According to the present results, using 2g yeast with 50 mg thyroxin/kg diet is the most viable option for optimizing growth and feed utilization in sea bream culture even when compared with diet containing 2g yeast. The present study is a preliminary study and further research is needed to determine the most appropriate supplement levels for optimum growth results in larger fishes at a commercial scale.

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اثر استخدام الخميرة واليود والثيروكسين في علائق اسماك الدنيس البحرية علي اداء الاسماك

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تقوم الدراسة علي بحث تأثير استخدام الخميرة منفردة كأضافة غذائية (بروبيوتك) وكذلك استخدامها مع اليود او هرمون الثيروكسين (سينبيوتكس) في علائق اصباغيات اسماك الدنيس البحرية علي معدلات نمو الاسماك وكفاءة الاستفادة من الغذاء والتركييب الكيميائي لاجسام الاسماك المرباة وكذلك دراسة تأثير هذه الاضافات علي بعض القياسات الجسدية وهي وزن الكبد ووزن الامعاء بالنسبة لوزن الجسم وكذلك معامل الحالة بالاضافة لقياس طول الاحشاء بالمقارنة بطول الجسم. ايضا تم دراسة اثر هذه الاضافات علي تركيب سيرم الدم حيث تم قياس البروتين والدهون الكلية وكذلك قياس هرمون الثيروكسين وانزيمي الليبيز والاميليز.

في البداية تم توزيع الاسماك في 12 حوض زجاجي بوزن ابتدائي $1,9 \pm 0,1$ جم مع استخدام 3 مكررات لكل معاملة وكانت المجموعة الاولى هي المجموعة الضابطة والتي تغذت علي الوجبة الاساسية بدون اضافات وتم تكوين 3 علائق للاختبار وكانت تحتوي العليقة المختبرة الاولى علي الخميرة كأضافة غذائية بنسبة 2 جم / كجم علف اما العليقة الثانية فأحتوت علي الخميرة بنفس نسبة الاضابة ومعها ايوديد البوتاسيوم بنسبة اضافته 0,01 جم / كجم علف اما العليقة الاختبارية الثالثة فكانت تحتوي علي 0,05 جم ثيروكسين بالاضافة للخميرة /كجم علف.

اظهرت النتائج ان افضل عليقة من حيث معدلات النمو وكفاءة الاستفادة من الغذاء والتركييب الكيميائي للاسماك في نهاية التجربة هي التي تحتوي علي الخميرة مع هرمون الثيروكسين كأضافتين في العليقة مقارنة باسماك المجموعة الضابطة يليها الاسماك التي احتوت وجبتها علي الخميرة منفردة كأضافة غذائية اما اسوأ النتائج فقد تم تسجيلها في مجموعة الاسماك التي احتوت الوجبة الخاصة بها علي اليود كأضافة في العليقة مع الخميرة

دلت القياسات الجسدية علي ان استخدام اليود قد ادي الي نقص في طول الاحشاء مقارنة بالمجاميع الاخرى من الاسماك اما تحاليل سيرم الدم فقد اظهرت ان اضافة اليود والثيروكسين في علائق الاسماك المرباة كان له تأثير معنوي علي كمية انزيمي الليبيز والاميليز في السيرم.