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Replacement of Fish Meal by Yeast (*Saccharomyces cerevisiae*): Effects on Digestibility and Blood Parameters for Gilthead Sea Bream (*Sparus aurata*)

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Abstract: A trial was conducted to test the effect of replacement of fishmeal by yeast, in isonitrogenous (48% CP) and isoenergetic (22 MJ kg^{-1}) diets for gilthead sea bream (*Sparus aurata)* with an initial average weight of 90±4 g. Diets were formulated to include 0% (control group), 10% (group I) and 20% (group II) from replacement of fish meal by yeast. Each diet was distributed by hand to satiation to triplicate groups of 25 fish per tank (1 m^3) and the growth trial lasted 12 weeks. Fish were adapted to study tanks and fed with control diet for one week. Fecal collection was began in the second week and samples were stocked in deep freeze (-20°C). Live weights of fish at the end of trial period were 140±11 g, 154±3 g and 157±8 for control group, group I and II, respectively. Growth rate and protein, lipid and cellulose digestibility were insignificant different among groups ($p>0.01$). Plasma glucose was found significant ($p<0.01$) but alkaline phosphates, Blood Urea Nitrogen (BUN), serum protein, cholesterol, triglyceride, albumin, amylase, GOT and GPT were not significant ($p>0.01$).

Key words: Yeast, replacement, fish meal, *Sparus aurata*, *Saccharomyces cerevisiae*

INTRODUCTION

Fishmeal is a limited feed resource and serious concern exists on the future availability of this feedstuff for incorporation in fish diets (Hardy, 1996; Sargent and Tacon, 1999). Yeast is one cell organism, which makes alcohol fermentation and break in sugar to alcohol. Yeast is an available feed ingredient for fish feed process. Besides it is easy to find and has lower price than many other ingredients. *Saccharomyces cerevisea* has several varieties as bread (bakery), beer and wine yeast. Bread yeast is easy to use at fish feed with its structure and nutritional composition (Reed and Peppler, 1973; Henrici, 1941).

Fishmeal is still the main or even the only protein source used in carnivorous fish diets, namely in marine fishes like sea bream. This is because fishmeal has the most adequate amino acid profile, is a very good source of essential fatty acids and minerals and is highly palatable (Oliva-Teles and Goncalves, 2001).

The evaluation of alternative protein sources to fishmeal is therefore a research priority. Among these, plant feedstuffs have received most attention in recent years; however, due to amino acid unbalances, presence of anti-nutritional factors and low palatability, a high level of replacement of fish meal with plant feedstuffs is generally not well accepted.

Single Cell Proteins (SCP) include micro algae, bacteria and yeast and are alternative protein sources that are used to feed ingredients for fish. SCP is insisting of different nutrient elements such as proteins, B-vitamins, pigments and complex carbohydrates and glucan (Sanderson and Jolly, 1994; Tacon, 1994). Especially, yeasts have been the most used within aqua feeds (Tacon, 1994). *Saccharomyces cerevisiae*, is believed to have immunostimulatory properties. Because of its complex carbohydrate components and nucleic acid content (Anderson *et al*., 1995).

Compared to fishmeal, the majority of the SCP are either deficient in one or more amino acids or they suffer from an amino acid imbalance (Tacon and Jackson, 1985; Kiessling and Askbrandt, 1993). The supplementation of yeast-based diets with the deficient amino acids was shown to have beneficial effects on fish growth (Nose, 1974; Bergstrom, 1979; Spinelli *et al*., 1979; Mahnken *et al*., 1980; Murray and Marchant, 1986).

In most monogastric animals, an excess of dietary nucleic acids supply is toxic, as the capacity of excretion of the uric acid formed is limited, leading to deposits of uric acid in the body and to possible disorders of metabolism (Schulz and Oslage, 1976; Tuse´, 1984). However, no such an effect was found in fish due to their very active liver uricase (De la Huiguera *et al*., 1981; Rumsey *et al*., 1991a).

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Tiews *et al*. (1979) reported to obtain with yeast-based diets supplemented with methionine growth rates equivalent to that of the control diet for rainbow trout. However, other attempts of using yeasts as the sole or the main dietary protein source resulted in reductions of fish performances (Beck *et al*., 1979; Mahnken *et al*., 1980; Rumsey *et al*., 1991a). Yeasts have been incorporated in rations at levels of 15-30%, resulting in a 25-50% replacement of the fishmeal content (Tacon and Jackson, 1985; Tacon, 1994).

The aim of this study is to compare the effect of replacing fishmeal with yeast at levels between 10 and 20% on the growth, digestibility and blood parameters of gilthead sea bream.

MATERIALS AND METHODS

This study was performed at the Ege University Fisheries Faculty Urla Laboratory and in Izmir, with gilthead sea bream (*Sparus aurata*), obtained from a commercial fish farm and consisted of a trial.

The trial is done with 9 cylindrical conic fiber digestive tanks (1 m^3) and marine water inlet by 32 mm PVC pipes, outlet by 50 mm PVC pipes. Feces collector is designed originally from PVC material. Each tank was stocked with 25 fish with an average weight of 90±4 g. To triplicate groups of these fish was distributed one of the experimental diets whose composition is presented in Table 1. Chromic oxide was included to the diets as an external marker. The fish were fed by hand to apparent visual satiety twice a day. Day light illumination is carried out. The trial lasted 12 weeks and fish were adapted to study tanks and fed with control diet for one week. Fecal collection was began in the second week and samples were stocked in deep freeze (-20°C). Chemical analyses of the diets, whole fish and feces were carried out as follows: dry matter after drying in an oven at 105°C until constant weight; ash by incineration in a muffle furnace at 450°C for 16 h; protein (N = 6.25) by the Kjeldahl method after acid digestion, using Kjeltec digestion and distillation units; lipids by petroleum ether extraction in a Soxtec HT System apparatus; chromic oxide of diets and feces by acid digestion according to Furukawa and Tsukahara (1966). Fish and feces were homogenized and dried before analyses. Blood samples were collected from the caudal vein and allowed to clot at room temperature for 4 h. After centrifugation, the serum was removed and frozen at -80°C, until used. Blood parameter of fishes was taken and analyzed by automatic analyst (Targu BT 3000 Plus-Biotecnica Instruments).

Formulations used at digestibility studies were as below (Hossu *et al*., 2003):

Table 1: Composition of experimental diets

Diet (DM%)	Control group	Group I*	Group II**
Fish meal	40.00	30.00	20.00
Yeast (*Saccharomyces cerevise*)	-	10.00	20.00
Soybean meal(CP48)	24.00	24.00	24.00
Wheat meal	20.30	20.30	20.30
Corn meal	6.00	6.00	6.00
Fish oil	9.00	9.00	9.00
Vitamin premix	0.30	0.30	0.30
Mineral premix	0.20	0.20	0.20
Binder	0.20	0.20	0.20
Proximate analysis (DM%)			
Crude protein	45.35	43.77	42.76
Crude lipid	8.89	7.60	7.82
Crude cellulose	3.01	2.59	2.51
Moisture	2.92	3.02	3.23
N free essential material	30.14	34.63	35.62
Ash	9.69	8.39	8.06
Gross energy (Kcal kg^{-1})	3437.80	3404.90	3411.10

*: 10% replacement by fish meal; **: 20% replacement by fish meal

$$\text{N free essential material (\%)} = 100\text{-[Crude (C) Protein + C Lipid + Ash + C Cellulose + Moisture]}$$

$$\text{Digestible energy (Kcal kg}^{-1}\text{)} = \text{Feed energy-Feces energy}$$

$$\text{Digestibility (\%)} = 100 - \left[\frac{\text{Indicator (feed\%)}}{\text{Indikator (feces\%)}} \times \frac{\text{Nutrient (feces\%)}}{\text{Nutrient (feed\%)}} \times 100\right]$$

In during, the trial period, water temperature averaged 19±0.5°C, salinity averaged 36±0.5%, dissolve oxygen averaged 8±0.5 mg L^{-1}, aeration was 2 L min^{-1} and pH was 7.5.

Statistical analysis consisted of one-way ANOVA and also blood analyses were subjected to Mann-Whitney test for two independent samples using Minitab-User Guide package programmer (Anonymous, 1993).

RESULTS

Feces were collected with collector and analyzed for inclusion of nutrients. Results of feces analyses were given Table 2. Crude protein levels are between 3.89 and 5.58 at the group I, 2.61-3.82 at control group and 1.82-3.51 at the group II. Crude lipid levels are between 0.09 and 0.40 at the group I, 0.28-1.49 at control group and 0.02-1.33 at the group II.

At the end of the trial, there were no significant differences in whole body weight among experimental groups.

According to the nutrient results of feed and feces, digestibility of feed is calculated and results are given at Table 3. Digestibility of feed for crude protein is average

Table 2: Results of feces analyses

	Group I			Control group			Group II		
Diets (%)	1	2	3	4	5	6	7	8	9
Moisture	78.97	76.17	71.51	77.62	75.88	79.31	86.71	85.59	70.11
C. protein	3.89	4.17	5.58	3.82	2.97	2.61	3.51	2.23	1.82
C. lipid	0.40	0.09	0.20	0.12	1.49	0.28	0.06	1.33	0.02
C. cellulose	1.64	1.86	1.95	0.99	1.84	1.58	1.59	0.92	1.88
N free E.M.*	10.4	12.61	15.62	13.35	12.02	12.72	3.23	4.83	21.39
Ash	4.70	5.10	5.14	4.10	5.80	3.50	4.90	5.10	4.80
Energy (kcal kg^{-1})	557.59	557.50	528.37	566.57	603.75	512.82	246.55	343.07	721.36

Table 3: Digestibility of feed ingredients (%)

	Group I			Control group			Group II		
Diets (%)	1	2	3	4	5	6	7	8	9
C. protein	91.11	90.47	87.25	91.58	93.45	94.24	91.79	94.78	95.74
C. lipid	94.73	98.82	97.37	98.65	84.24	96.85	99.23	82.99	99.74
C. cellulose	36.68	28.19	24.71	67.11	38.87	47.51	36.65	63.35	25.10
N free E.M.*	65.49	58.16	48.17	61.44	65.29	63.26	90.93	86.44	39.94
Energy	83.78	83.78	84.63	83.36	82.26	84.93	92.77	89.94	78.85

*: Nitrogen free essential material

89.61, 93.09 and 94.10 at group I, control group and group II, respectively. Digestibility of crude lipid is average 96.97 at group I, average 93.24 at control group and average 93.98 at group II.

Plasma glucose level was average 62.2±8.5 mg dL^{-1}, average131±43.5 mg dL^{-1} and average 74.4±9.5 mg dL^{-1} at group I, group II and control group, respectively. Other blood parameters; which is alkaline phosphates, Blood Urea Nitrogen (BUN), serum protein, cholesterol, triglyceride, albumin, amylase, GOT (Glutamate oxalacetate transaminaze) and GPT (Glutamate pyruvate transaminaze), were not significant ($p>0.01$).

DISCUSSION

Feed conversion of sea bream improved with the replacement of 20% fishmeal by yeast. At the end of trial period; body weights of fish were found 140±11, 154±3 and 157±8 g at control group, group I and II, respectively. Oliva-Teles and Gonçalves (2001) reported that feed conversion of sea bass improved with the inclusion of up to 30% dietary protein from brewers yeast. There were no significant differences in growth performance with the replacement of 50% of fishmeal protein by brewer's yeast, the maximum level tested in their study. Also same results in sea bass, Metailler and Huelvan (1993) tested the inclusion of 10, 20 and 30% of lactic yeast, bakers yeast and brewers yeast in isoproteic fishmeal-based diets. The researchers found no differences in growth and feed utilization among groups, except for fish fed diets including brewers yeast. Alliot *et al.* (1979) found no negative effect on growth performance of sea bass fed diets with 50% dietary fishmeal protein replaced by an alkane yeast protein. In the study, fish feed diets with >20% replacement of fishmeal by yeast growth performed better than those fed other diets. However, there were no significant differences between groups in growth performance ($p>0.01$).

In the study, protein, nitrogen free essential material and energy digestibility in group II were higher than other groups but lipid digestibility in group I was higher than other groups. Also, cellulose digestibility in control group was higher than other groups. Rumsey *et al.* (1991b) found that digestibility of intact brewers yeast in rainbow trout is significantly lower than that of disrupted cells. In rainbow trout, both true and apparent digestibility coefficients of brewers yeast protein were estimated to be lower than in sea bass (Atack and Matty, 1979; Rumsey *et al.*, 1991b). Olvera-Novoa *et al.* (2002) reported that it is possible to replace up to 65% of animal protein with a mixture of plant proteins, including 30% from torula yeast, in tilapia fry diets without adverse effects on fish performance and culture profit. Alcohol extraction removes oligosaccharides.

Extrusion at high temperatures can also improve digestibility of polysaccharides because of a higher breakup of cell walls and partial degradation of a-galactosides (Alexis and Nengas, 2001). Protease enzyme is inside the live yeast cell. By the beginning of age (inactivation) some cells are die and protease enzyme is revealed. Yeast in experimental feeds were inactive, old bread yeast, which mostly existences by death cells (Sultan, 1986). This might effects the digestibility of protein positively, which might very important in commercial scale. In this study, there were no significant differences between groups in protein digestibility, lipid digestibility, cellulose digestibility, nitrogen free essential material digestibility and energy digestibility ($p>0.01$).

Plasma glucose levels were found 62.2±8.5, 131±43.5 and 74.4±9.5 mg dL^{-1} in group I, group II and control group, respectively. Plasma glucose value was significant between groups ($p<0.01$). Glucose level might effect from water temperature, pH, stress, feed composition and feeding methods (Hemre *et al*., 1996). Glucan at cell wall of yeast occur combination of sugar molecules. This might force to rise of glucose level in plasma (Navvaro *et al*., 1993; Kaminska *et al*., 1998). Insulin, glucagons and thyroid hormones are regulator of glucose metabolism at mammalians. Even, if it is not proved at fishes this might slow down the body metabolism which might cause weight gain.

Alkaline phosphates, Blood Urea Nitrogen (BUN), serum protein, cholesterol, triglyceride, albumin, amylase, GOT (Glutamate oxalacetate transaminaze) and GPT (Glutamate pyruvate transaminaze) were no significant ($p>0.01$).

CONCLUSION

It seems that while looking for alternative feed ingredients, yeast might be one these. Oliva-Teles and Gonçalves (2001) were study with bread yeast for sea bass juveniles. Replacement percentage might be higher by heating of yeast before processing. Results are promising for replacement of yeast by fish meal for gilthead sea bream.

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