

Growth and feed utilization of goldfish (*Carassius auratus*) fed graded levels of brewers yeast (*Saccharomyces cerevisiae*)

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Received: October 2014

Accepted: March 2016

Abstract

In this study, a feeding trial was conducted to examine the potential of replacing fish meal with brewers yeast in practical diet of goldfish (*Carassius auratus*). Five isoproteic (37% CP) and isocaloric (3350 kcal/kg) diets were formulated to contain graded levels of brewers yeast. Fish meal protein was replaced by 0%, 15%, 25%, 35%, and 45% of yeast. Each diet was randomly allocated to triplicate groups of 20 fish (initial average weight of 0.56 g fish⁻¹) in glass aquarium (65L). Fish were fed three times per day to apparent satiation for 84 days. At the end of the experiment, weight gain, specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (CF), survival rate (SR), hepatosomatic indices (HSI) and body composition of goldfish fry were determined. According to the results, weight gain, SGR, FCR and PER of fish fed the diet including yeast replaced 35% of the fish meal were better than those of fish fed the other diets. There were no significant differences in SR and HSI values among fish fed diets ($p>0.05$). However, CF among fish fed the experimental diets was significantly differ ($p>0.05$). Whole body composition was similar among fish fed different diets. The optimal replacement level of fishmeal protein by brewers yeast was determined by second-order polynomial regression to be ($y= 2, 2237- 0,0004x^2 + 0,0279x$; $R^2 = 0,9977$) 34.875%, on the basis of SGR.

Keywords: *Saccharomyces cerevisiae*, *Carassius auratus*, Fish meal replacement, Growth, Feed utilization.

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Introduction

Ornamental fish has become increasingly important today all around the world. The ornamental fish trade in the world is to have a volume of US \$1 billion annually (FAO, 2010). Ornamental fish sector has consisted of marine and freshwater ornamental fishery. One of the most important species of freshwater ornamental fishery is goldfish, *Carassius auratus*. It is a kind of fish that widely preferred by hobbyists because of species diversity, attractive and variety of colors, and high tolerance to environmental demands. The healthy and fast growing individuals for goldfish are very important to be met of market demand. In this reason, goldfish rearing is very important to feed diet with balanced energy/protein ratio and low price.

Fish meal as a major source of protein, being high nutritional value and taste are preferred in fish feed preparation. However, various researches are made as to the use of cheaper alternative protein sources to replace partially or completely of fish meal due to the expensive raw material and difficulties experienced in the production of fish meal (Tacon and Metian, 2009). One of alternative protein sources in aquafeeds is single cell protein micro algae, bacteria and yeast (Tacon, 1994). Among them, the yeasts are important species including *Candida* sp, *Hansenula* sp, *Pichia* sp. and *Saccharomyces* sp. Brewers yeast (*S. cerevisiae*) is a natural product from the brewing industry that contains rich in protein, various immunostimulating compounds such as B-glucans, nucleic

acids as well as mannan oligosaccharides (Li and Gatlin, 2003; Ghosh *et al.*, 2005; Abdel-Tawwab *et al.*, 2008; 2010), and has been used as a diet additive for various animals. It has been observed to be capable of enhancing growth (Lara-Flores *et al.*, 2003; Li and Gatlin, 2003; Ebrahim and Abou-Seif, 2008; Zerai *et al.*, 2008; Korkmaz and Çakıroğulları, 2011; Guroy *et al.*, 2012; Omar *et al.*, 2012) and immune responses (Ortuno *et al.*, 2002) of various fish species.

The studies on using brewers yeast for growth performance of goldfish have not been adequate. Therefore, the present study was to evaluate the effect of partial replacement of fish meal in the diet with brewers yeast (*Saccharomyces cerevisiae*) on growth, feed utilization and carcass composition of goldfish fingerlings.

Materials and methods

Diet preparation

Five experimental diets were formulated to 37% crude protein, 8% crude lipid and 3350 kcal/kg digestible energy with 0%, 15%, 25%, 35%, and 45% of brewers yeast (Table 1). Fishmeal and soybean meal were provided by the Abalioglu Feed-Soybean and Textile Industries, Inc., Denizli, Turkey. Brewer's yeast was obtained from private beer producing Ltd. All ingredients were thoroughly mixed, and 400 mL of water was added per kg diet.

Table 1: Formulation and proximate analysis of the experimental diets (% dry matter basis) fed to goldfish (*Carassius auratus*) fry

Ingredients (%)	% yeast replaced by FM				
	0	15	25	35	45
Fish meal	40	34	30	26	22
Brewers yeast ¹	0	8.87	14.78	20.71	26.63
Soybean meal	20	20	20	20	20
Corn starch	25.15	21.93	20.12	19.19	17.17
Fish oil	2.75	3.6	4	4.5	5
Vitamin premix ²	3	3	3	3	3
Mineral premix ³	3	3	3	3	3
Methionine	1	1	1	1	1
NaCl ⁴	0.1	0.1	0.1	0.1	0.1
CaHPO ₄ ·2H ₂ O ⁵	1	1	1	1	1
CMC ⁶	1	1	1	1	1
Cellulose	3	2.5	2	0.5	0.1
Total	100	100	100	100	100
Proximate analysis (% wet weight basis) ⁷					
Dry matter	94.99±0.04	95.09±0.01	95.01±0.01	95.05±0.06	95.00±0.01
Crude protein	37.31±0.05	37.44±0.03	37.39±0.05	37.37±0.03	37.49±0.05
Crude lipid	8.01±0.01	7.96±0.04	7.95±0.02	7.92±0.04	7.99±0.04
Crude ash	4.76±0.01	2.93±0.40	3.44±0.50	3.43±0.42	3.06±0.28
Energy (DE kcal/kg) ⁸	3443	3381	3336	3328	3285

¹Brewers yeast (*Saccharomyces cerevisiae*)

²Vitamin premix (per mg/kg feed, except where indicated); Vitamin A Retinol, 4 000 000 IU; Vitamin D₃, 600 000 IU; Vitamin E Tocopherol, 40 000; Vitamin K₃ Menadione, 2 400; Vitamin B₁, 5 000; Vitamin B₂, 8 000; Vitamin B₆, 4 000; Vitamin B₁₂, 12; Vitamin C, 40 000; Niacin, 50 000; Folic acid, 1400; D-calcium Pantothenate, 8 000; D-Biotin, 50; and Inositol, 40 000.

³Mineral premix (per mg/kg feed); MnSO₄, 60 000; FeSO₄, 10 000; ZnSO₄, 75 000; CuSO₄, 5 000; CoSO₄, 1 000; KIO₃, 2 500; NaSeO₃, 100; and MgSO₄, 65 000.

⁴Sodium chloride, ⁵Calcium hydrogen phosphate, ⁶Carboxy-metil cellulose, ⁷Values are mean (±SE) of three replicate. ⁸Digestible energy calculated as protein 4.9 kcal g⁻¹, lipid 9.01 kcal g⁻¹ and carbohydrate 3.49 kcal g⁻¹ (NRC, 1993).

Then the mixture of each diet was separately passed through a meat grinder, and pelleted through 2-mm-diameter diet. The pellets were dried in a drying oven for 24 hours at 50°C, crushed into desirable particle sizes, then sealed in bags and stored in frozen at -20°C prior to use in the feeding experiment. The dietary composition and chemical analyses of the experimental diets are shown in Table 1.

Fish and Experimental conditions

This study was conducted from March 2 through May 25, 2012 at Aquaculture Experiment Unit, Akdeniz University, Antalya. Goldfish fry were obtained from local ornamental fish farm in Antalya Province. After two weeks acclimation, fish were randomly distributed at 20 fish with initial mean weight 0.56±0.01g to each 65L glass aquarium. Each treatment was run in triplicate. A 100g sample of fish was frozen at -20°C for initial proximate analysis. Each aquarium was supplied with compressed air by air stone using

central air pump. Half of the aquarium water was siphoned daily for removing fish wastes, and water volume was replaced by aerated water from the storage tank. A 12h light: 12h dark photoperiod was maintained with fluorescent lights controlled by timers, and light intensity at the water surface of the aquaria was kept constant as 150 lux. Fish in all treatments were hand-fed to visual satiation three times a day at 9:00, 13:00 and 16:00 and feed intake was recorded daily for 12 weeks. Dead fish were recorded daily and removed from each aquarium. Water quality parameters were monitored and maintained during the experiment. Dissolved oxygen and temperature were measured on site using a WTW multi-meter. pH was measured using a pH-meter (WTW, Wissenschaftlich-Weilheim, Germany).

Growth parameters

At the end of the experiment, before weighing, fish were anesthetized using clove oil (v:v, 1/20) to reduce their stress. Fish were individually weighed and measured. Five fish from each aquarium were dissected, and the liver and viscera weighed. Fish growth parameters and feed utilization were calculated as follows (Gümüş *et al.*, 2013):

Weight gain (WG, g)= $W_f - W_i$; Specific growth rate (SGR, %/day)= $100 \times (\ln W_f - \ln W_i) / \text{days}$, where W_i and W_f are the initial and final weights (g), respectively.

Feed conversion ratio (FCR)=Feed intake (g)/WG; Protein efficiency ratio

(PER)=WG/Protein intake (g); Condition Factor (CF) = $100 \times [(\text{final fish weight (g)} / \text{final fish length (cm)})^3]$; Hepatosomatic indices (HSI, %)= $100 \times (\text{liver weight (g)} / W_f \text{ (g)})$; Survival rate (SR, %)= $100 \times (\text{Final number of fish} / \text{Initial number of fish})$.

Chemical analysis

Proximate composition of experimental diets and fish samples were analyzed according to standard methods of Association of Official Analytical Chemists (AOAC, 1995). Dry matter was estimated by drying samples to constant weight at 105°C in a drying oven. Crude protein determined by Kjeldahl method ($N \times 6.25$) after acid digestion, crude lipid by ether extraction using Soxtec system, and ash by incineration at 550°C for 24 h.

Statistical analysis

Data were subjected to one-way analysis of variance (ANOVA). Differences between means were tested at the 5% probability level using Duncan's multiple-range test. Results were presented as mean±SE. All statistical analyses were performed using SPSS software 15.0 (SPSS Inc., Chicago, IL, USA). A second-order polynomial regression analysis method was conducted to analyze of the relationship between SGR and the replacement levels of fish meal protein by brewers yeast in the diets of goldfish.

Results

The parameters for water quality throughout feeding experiment were recorded for temperature were in the range of 24-28°C, dissolved oxygen from 5.00 to 6.23 mg/L, and pH from 7.71 to 7.89. Water quality parameters were maintained within the acceptable ranges for goldfish (Lochmann and Phillips, 2002).

The growth performance, feed efficiency and survival rate of goldfish fed the experimental diets are shown in Table 2. Goldfish fed the diets supplemented with brewers yeast tended to have better growth performance during the 12-week feeding experiment. Final fish weight, weight gain and specific growth rate for fish fed 35% brewers yeast were higher than those of the other groups ($p < 0.05$). The relationship between dietary brewers yeast levels and the SGR was

the best described by the second-order polynomial regression equation as follows: $Y = 2,2237 - 0,0004x^2 + 0,0279x$. According to differential equation, maximum SGR occurred at brewer yeast level of approximately 34.875% (Fig. 1).

Feed conversion ratio and protein efficiency ratio were improved by increasing dietary levels up to 35% brewers yeast. However, condition factor was tended to decrease in fish fed diets inclusion graded levels of brewers yeast ($p < 0.05$).

Hepatosomatic index and survival rates were not significantly different among the different treatments. No significant differences also were found in whole-body dry matter, protein, lipid or ash contents of fish fed the different experimental diets ($p > 0.05$) (Table 3).

Table 2: Growth and feed utilization of goldfish (*Carassius auratus*) fed with experimental diets.

Parameters (%)	% yeast replaced by FM				
	0	15	25	35	45
Initial body weight (g)	0.56±0.00	0.56±0.00	0.56±0.00	0.56±0.00	0.56±0.00
Final body weight (g)	3.64±0.10 ^c	4.79±0.12 ^b	5.25±0.34 ^{ab}	5.51±0.16 ^a	5.26±0.16 ^{ab}
Weight gain (g/fish)	3.08±0.10 ^c	4.24±0.12 ^b	4.69±0.34 ^{ab}	4.96±0.16 ^a	4.70±0.16 ^{ab}
SGR (% day ⁻¹)	2.22±0.02 ^c	2.55±0.03 ^b	2.65±0.07 ^{ab}	2.72±0.03 ^a	2.66±0.03 ^{ab}
FCR	3.32±0.18 ^c	2.68±0.03 ^b	2.42±0.18 ^{ab}	2.26±0.03 ^a	2.34±0.04 ^{ab}
PER	0.68±0.04 ^c	0.86±0.01 ^b	0.97±0.08 ^{ab}	1.04±0.01 ^a	1.01±0.01 ^{ab}
CF	1.79±0.00 ^a	1.83±0.01 ^a	1.65±0.10 ^{ab}	1.67±0.03 ^{ab}	1.54±0.05 ^b
HSI (%)	2.98±0.01	3.18±0.42	2.85±0.13	2.44±0.30	2.96±0.41
Survival (%)	95.00	96.67	96.67	96.67	98.33

Values are mean (±SE) of three replicate. Values with the same superscripts within the same row are not significantly different ($p > 0.05$).

Table 3: Chemical composition of goldfish (*Carassius auratus*) fed with experimental diets.

Parameters (%)	% yeast replaced by FM				
	0	15	25	35	45
Crude protein	15.32±0.04	15.70±0.40	16.09±0.24	16.12±0.05	15.97±0.16
Crude lipid	5.48±0.05	5.21±0.31	5.19±0.35	5.28±0.42	5.44±0.24
Crude ash	3.34±0.31	2.78±0.22	3.40±0.01	2.86±0.11	3.06±0.12
Dry matter	24.72±0.44	24.25±0.32	25.19±0.01	24.70±0.30	24.64±0.20

Values are mean (±SE) of three replicate.

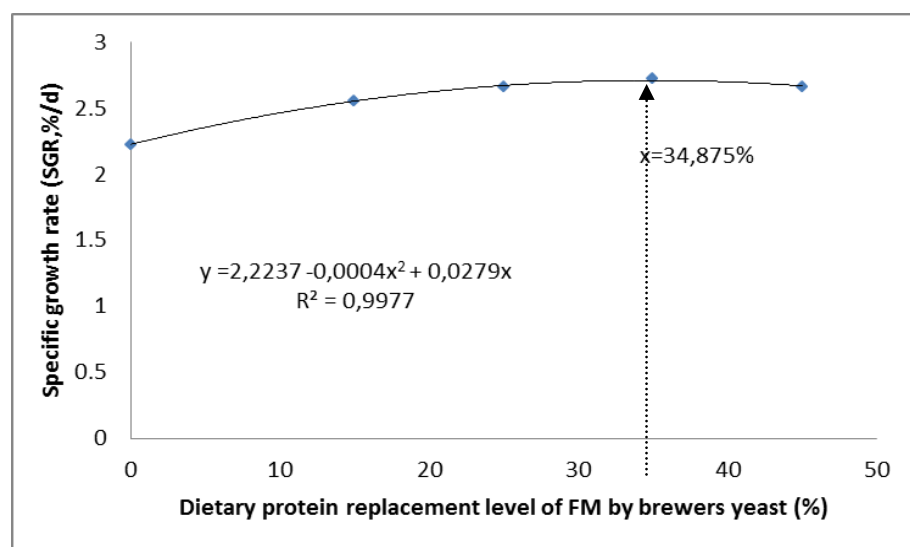


Figure 1: Relationship between specific growth rate (SGR) and dietary compound yeast supplementation level for goldfish (*Carassius auratus*) as described by second-order polynomial regression.

Discussion

The present study indicated that brewers yeast was replaced up to 35% of fish meal protein in the diets and improving growth and feed utilization. However, inclusion of brewers yeast above 35% fish meal protein replacement resulted in a slightly decrease in growth performance of goldfish.

The nutritional value of brewers yeast has been tested to affect on growth performance and feed utilization in many fish species such as Nile tilapia (Lara-Flores *et al.*, 2003; Abdel-Tawwab *et al.*, 2008; Korkmaz and

Cakirogullari, 2011; Asadi Rad *et al.*, 2012), rohu (Tewary and Patra, 2011), rainbow trout (Rumsey *et al.*, 1991; Guroy *et al.*, 2012), carp (Omar *et al.*, 2012), red drum (Li and Gatlin, 2005), beluga (Hoseinifar *et al.*, 2011), and sea bass (Oliva-Teles and Goncalves, 2001). The results were obtained that supplementation with 25% brewers yeast for rainbow trout (Rumsey *et al.*, 1991), 50% brewers yeast for sea bass, *Dicentrarchus labrax* (Oliva-Teles and Goncalves, 2001), 30% yeast for koi carp (Korkmaz and Cakirogullari, 2011), 27-41% bio-ethanol yeast for sunshine, *M. chrysops* × *M. saxatilis*,

(Gause and Trushneski, 2011), 50% yeast for carp (Omar *et al.*, 2012), 30% organically certifiable yeast for rainbow trout (Guroy *et al.*, 2012), and 40% brewers yeast for Atlantic salmon (Overland *et al.*, 2013). Similar pattern that improved growth performance in tilapia fed yeast diets have been reported by Olvera-Novoa *et al.* (2002), Lara-Flores *et al.* (2003), Li and Gatlin (2005), Abdel-Tawwab *et al.* (2008), Zerai *et al.* (2008), Osman *et al.* (2010), and Ozorio *et al.* (2012).

Polynomial regression has been recommended for describing and approximating the relationship between SGR and dietary brewers yeast levels. In the present study, the second-order polynomial regression analysis has been suggested that the optimal replacement level of fish meal protein was about 34.875% (Fig. 1). Based on this analysis, no significant improvements of weight gain or SGR were observed in goldfish fed higher concentration of the brewers yeast supplemented in the diets.

The better FCR and the higher PER values were observed in fish that fed up to 35% brewers yeast supplemented diets which improved feed utilization of goldfish. Oliva-Teles and Gonçalves (2001) found better feed efficiency when 30% of the fish meal protein was replaced with brewers yeast for sea bass. At the end of the experiment, fish survival was high and ranged from 93% to 98% without significant difference among treatments ($p>0.05$). Also no significant differences were observed in

condition factor and HSI values of goldfish fed the experimental diets.

Whole-body dry matter, crude protein, crude lipid, and ash content were not affected by the different dietary treatments (Table 3). These results took the same trend of those results obtained by Olvera-Novoa *et al.* (2002), that they observed no differences in carcass composition when substituting fish meal protein with torula yeast in diet for tilapia fry. Ebrahim and Abou-Seif (2008) reported that carcass composition was not affected by dietary brewers yeast ($p>0.05$). Moreover, Guroy *et al.* (2012) reported that use of organically certified yeast did not change the whole-body contents of rainbow trout. According to the Gaese and Trushenski (2011) there were no significant differences in proximate composition, but lipid composition demonstrated decrease fish fed with all yeast supplemented diets. Oliva-Teles and Gonçalves (2001) and Hunt *et al.* (2013) reported that protein content which was significantly higher in fish fed diets containing yeast than the control diet. In contrast with our findings, rainbow trout fed the diets containing yeast showed decreased whole body protein lipid when compared with control diet (Hunt *et al.*, 2013).

In conclusion, this study suggested that brewers yeast could replace 35% of fish meal protein in the diet of goldfish without any adverse effect on growth performance and feed utilization, but an optimal replacement level of 34.875% is suggested.

Acknowledgements

The authors would like to thanks M. Emin Akdoğan and Gizem Ülker for their helps throughout the period of the study.

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