



## Evaluating Two Different Additive Levels of Fully Autolyzed Yeast, *Saccharomyces cerevisiae*, on Rainbow Trout (*Oncorhynchus mykiss*) Growth Performance, Liver Histology and Fatty Acid Composition

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### Abstract

This study examined the effects of fully autolyzed *Saccharomyces cerevisiae* (ASC) on rainbow trout (*Oncorhynchus mykiss*) growth performance, liver histology and fatty acid composition. 2700 rainbow trout (4.9 ±0.1 g mean weight) were acclimated to rearing conditions and then randomly divided into three groups of triplicate cages. Rainbow trout were fed with three different doses of ASC (0 (control), 1 ‰ and 2 ‰) for 70 days. At the end of the study, growth performance, liver histology, fatty acid composition and survival rate were determined. Feed conversion ratio was significantly decreased and specific growth rate was increased (P<0.01) on 2‰ ASC added group compared to other groups. No differences were observed among group's liver histology. Whole-body proximate composition showed no differences among groups (P>0.05) except in fat content, which was enhanced in both ASC groups compared to control (P<0.05). The fatty acid composition of fish fillets differed only marginally among the experimental groups. The muscles of fish fed the diets with ASC had a significantly decreased in 18:0 (P<0.05) and an increased in 15:1 (P<0.01) content compared to control groups. The content of n-3 fatty acids and DHA/EPA ratio were not affected by ASC addition. In conclusion, the results of the study indicate that 2‰ supplementation level of the ASC showed beneficial effects on the growth performance without affecting moisture, protein and ash content of rainbow trout.

Keywords: Autolyzed *Saccharomyces cerevisiae*, Rainbow Trout, Growth Performance, Fatty Acid Composition, Liver Histology.

### Introduction

Trout is major cultured finfish species in Turkey and it has reached its maximum production in 2015 with 108090 tons. Moreover, trout production in Turkey is continually increasing in conjunction with developing technologies and their application onto fish farms. However, the higher disease susceptibility of the cultured organism due to deterioration of water quality and elevation of the other stressful conditions emerged even though the more intensification of fish production is achieved. (Bondand-Reantaso *et al.*, 2005).

There are some limiting factors in aquaculture such as fish diseases, which is one of the main problem in aquaculture, and antibiotic practices are a common approach to resolve these problems (Austin and Austin, 1989). However the antibiotic usage have been limited in many countries due to increasing bacteria resistance against antibiotic and residues on the carcass (Smith *et al.*, 1994). Likewise the existence of residues in aquaculture products menaces

human health. Nowadays, the use of probiotics and prebiotics are being industrialized to control diseases and a wholesome microbial environment in aquaculture systems could sustain (Verschuere *et al.*, 2000; Bachère 2003; Defoirdt *et al.*, 2005, Apun-Molina *et al.*, 2009).

Probiotic is defined as any microbial adjunct that has beneficial consequences on the host through an enhancement in the use of feed or by enhancing the host reaction to disease (Irianto and Austin, 2002; Kesarcodi-Watson *et al.*, 2008; Abdel-Tawwab *et al.*, 2008). There are some different probiotic species such as *Bifidobacterium* sp., *Lactobacillus* sp., *Enterococcus* sp., *Vibrio* sp., *Saccharomyces* sp., and *Bacillus* sp, which are currently used in aquaculture (Kumar, Mukherjee *et al.*, 20016).

*Saccharomyces cerevisiae* (Baker's yeast), contain numerous immunostimulant complexes such as chitin, β-glucan, mannan oligosaccharides and nucleic acids. All those natural products have the capability to improve growth performance of various fish species (Gopalakannan and Arul 2010).

Effects of nucleic acids or  $\beta$ -glucan in finfish were studied previously (Li and Gatlin, 2003; Wache *et al.*, 2006; Chiu *et al.*, 2010; Gopalakannan and Arul, 2010). In these investigations, some factors such as growth performance, disease resistance and blood immune response were reported. However, there is no published information regarding the effects of completely autolyzed *Saccharomyces cerevisiae* (ASC) on histological changes and fatty acid composition in rainbow trout tissues. Therefore, this study was performed to investigate the effect of dietary ASC on rainbow trout growth performance, fillet fatty acid composition and liver histology.

## Materials and Methods

### Diet Preparation

ASC was added to the commercial fish feed (Skretting) at two doses 1‰ and 2‰ served as treatments (Ustaoğlu Güven, 2010) (Table 1). The diet containing no supplementation was control and all these prepared feed was stored at 4°C until they were used. The ASC was diluted into 1% sunflower oil, thoroughly homogenized and added to feed. The same volume of oil (without the ASC) was added to the control diet.

### Fish and Feeding Trial

Juveniles rainbow trout (average individual weight of  $4.9 \pm 0.1$  g) were provided from Experimental Animal Center of Kastamonu University, Turkey. The experiment was performed in Germeçtepe Dam Lake, in three replicates for each treatment, and two thousand and seven hundred fish were allocated into 2 m<sup>3</sup> fish cages (600 fish/cage) equally. The fish were fed with the experimental diets *ad-libitum* three times a day for 70 days. Throughout the experiment, water quality parameters were measured daily and as average dissolved oxygen was  $7.41 \pm 0.2$  mg/L, temperature was  $17.4 \pm 0.1$  °C, pH was  $7.3 \pm 0.2$ .

### Histopathological Examination

Changes on liver histology of rainbow trout were examined on the 70<sup>th</sup> day of the experiment. 9 fish from each cage were euthanatized by overdose of MS-222 for organs collection at the end of the feeding trial. Organs were carefully removed, immediately

fixed in a 10% buffered solution of formalin, dehydrated in a graded ethanol series, and embedded in paraffin. Sections were cut at 5  $\mu$ m and stained with hematoxylin and eosin for light microscopic investigation. Inflammation, damage or regeneration of tissue, and nuclear position of organs sections were tested for general abnormalities (Hisar *et al.*, 2002).

### Proximate and Fatty Acid Composition

Crude protein, lipid, moisture and ash of the experimental feed and fish muscles were determined by methods as defined in A.O.A.C. (2000) methods. Determination of fatty acid composition of lipids from the fillet of rainbow trout tissues were maintained with a mixture of chloroform and methanol (2:1, v/v; (Folch *et al.*, 1957) containing 0.01% BHT. Lipid extracts were stored at -20 °C prior to analysis. Fatty acid compositions of the fishes were determined according to Miller *et al.* (2007).

### Growth Performance

At the end of the study, all fish in each tank were individually weighted, and 9 fish from each replicates were used to investigate production performance. Growth parameters were computed as follows: Feed Conversion Ratio (FCR) = feed intake (g) / weight gain (g) x 100. Specific Growth Rate (SGR) (% day<sup>-1</sup>) =  $100 \times [(\ln \text{ final fish weight}) - (\ln \text{ initial fish weight})] / \text{days fed}$ . Feed Intake (FI) (%) = daily feed intake (g) x 100 / biomass (g) Protein Efficiency Ratio (PER) = wet body mass gain/crude protein intake (Wojno, 1977; Laird and Needham 1987; Fowler 1991) .

### Statistics

Statistical analyses were performed using one-way ANOVA. For multiples comparisons of the means Tukey-Kramer HSD was used. All the results were treated significant at the 5% and 1% level. All statistical analysis was performed using the SPSS software version 17.

## Results

The growth parameters, feed conversion rate and survival rate of the fish were presented in Table 2. No significant differences were observed on survival rate of different experimental groups.. The final

**Table 1.** Proximate composition of experimental feed. Values are provided as mean  $\pm$  standard error

	Commercial Feed
Moisture (g kg <sup>-1</sup> )	9 $\pm$ 0.24
Protein (g kg <sup>-1</sup> )	45 $\pm$ 0.11
Fat (g kg <sup>-1</sup> )	20 $\pm$ 0.23
Ash (g kg <sup>-1</sup> )	4.1 $\pm$ 0.49
Cellulose	3.9 $\pm$ 0.78

weights, specific growth rates and feed conversion ratio of fish fed with 2% ASC were significantly improved.

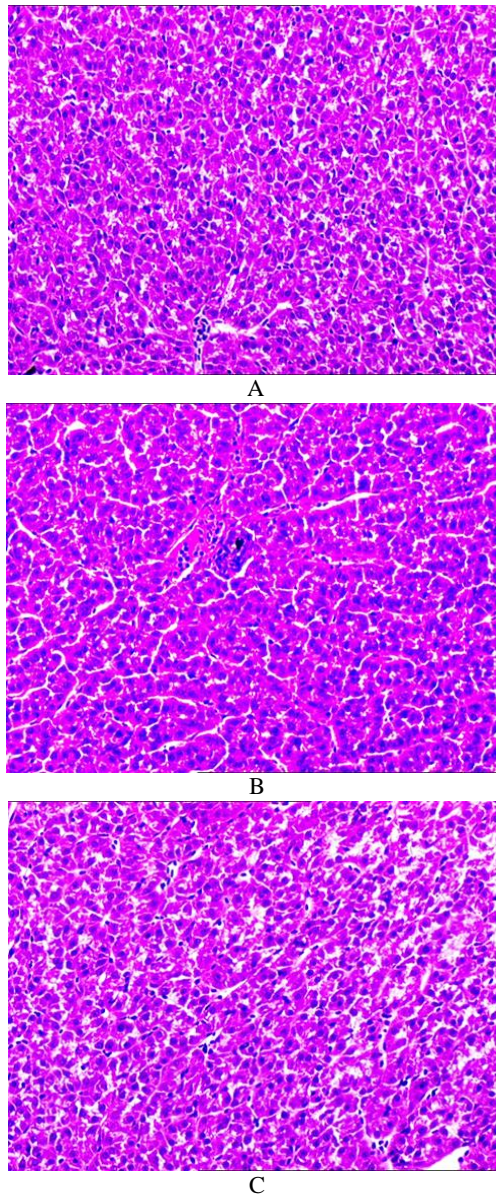
Histological analysis did not show any

pathological changes on livers of the fish (Figure 1). Muscle proximate composition analysis (Table 3) also uncovered that, crude protein, moisture and ash were unaffected ( $P < 0.05$ ) in the trial. However the total

**Table 2.** Growth performance of fish fed experimental diets

	Diets		
	Basal Diet (Control)	Diet 1 (added %1 <i>S. cerevisiae</i> )	Diet 2 (added %2 <i>S. cerevisiae</i> )
Initial weight (gr)	4.96±0.10	4.88±0.10	4.88±0.10
Final weight (gr)	14.90±0.40 <sup>a</sup>	14.52±0.70 <sup>a</sup>	18.46±0.30 <sup>b</sup>
Feed conversion ratio (FCR)	1.20±0.10 <sup>a</sup>	1.22±0.10 <sup>a</sup>	0.86±0.09 <sup>b</sup>
Specific growth rate (% day <sup>-1</sup> )	1.90±0.10 <sup>a</sup>	1.88±0.10 <sup>a</sup>	2.22±0.09 <sup>b</sup>
Survival	87.50±8.80	70.00±6.60	86.67±10.60
Growth (%)	311.93±15.90 <sup>a</sup>	309.37±10.60 <sup>a</sup>	378.82±8.42 <sup>b</sup>

Values are provided as mean ± standard error (n=10). Values with different superscripts in horizontal row are significantly different ( $P < 0.05$ ).



**Figure 1.** Histological findings in fish treated with different doses of ASC and control (A: Control B: %1 C: %2).

lipid level in fish fed with 1‰ and 2‰ ASC were significantly increased.

Fatty acids composition of fish muscles were (Table 4) slightly influenced by dietary treatments. Fatty acid 18:0 ( $P<0.05$ ) was significantly decreased in all experimental groups compared to control and 15:1 ( $P<0.01$ ) was significantly elevated in 2‰ ASC group. The totals of n-3 fatty acids did not differ among the three experimental groups and neither were any differences seen in total of n-6 fatty acids or in any other totals of fatty acids including saturated [SFA], monounsaturated [MUFA] and polyunsaturated [PUFA] fatty acids. Furthermore, rations of docosahexaenoic acid (22:6 n-3; DHA) to eicosapentaenoic acid (20:5 n-3; EPA) were also very similar and non-significant among the fish muscles.

## Discussion

In the current study, ASC was tested as feed

additive for growth and liver histology in rainbow trout. It was indicated that ASC added at 2‰ to fish feed was effective for positive growth promotion. However, any positive or negative effects of ASC have not been observed on liver histology. At this point, growth promotion may enable and render a better growth in the rainbow trout.

In recent years, in aquaculture, there has been great attention in the use of brewer's yeast as a probiotic owing to brewer's yeast have been indicated to modulate intestinal microbiota and affect growth promotion positively in different fish species (Abdel-Tawwab *et al.*, 2008; Hoseinifar *et al.*, 2011; Goda *et al.*, 2012). In the present study, fish fed diet with 2‰ level of ASC showed a significant decreasing on FCR and increasing SGR level even though dietary ASC supplementations did not significantly affect survival rate. The improved growth and feed utilization observed in the present study are in agreement with those reported by Diab *et al.* (2006), who fed Nile

**Table 3.** Proximate composition of whole-body rainbow trout fed with experimental diets for 70 days. Values are provided as mean  $\pm$  standard error (n=10 per treatment). Values with different superscripts in row are significantly different ( $P<0.05$ )

	Diets		
	Basal Diet (Control)	Diet 1 (added 1‰ <i>S. cerevisiae</i> )	Diet 2 (added 2‰ <i>S. cerevisiae</i> )
Moisture (g kg <sup>-1</sup> )	73.02 $\pm$ 0.10	71.67 $\pm$ 0.09	72.19 $\pm$ 0.51
Protein (g kg <sup>-1</sup> )	19.87 $\pm$ 0.10	19.98 $\pm$ 0.10	19.78 $\pm$ 0.03
Fat (g kg <sup>-1</sup> )	4.78 $\pm$ 0.10 <sup>a</sup>	6.79 $\pm$ 0.07 <sup>b</sup>	6.16 $\pm$ 0.41 <sup>b</sup>
Ash (g kg <sup>-1</sup> )	2.33 $\pm$ 0.10	2.29 $\pm$ 0.06	2.27 $\pm$ 0.04

**Table 4.** Percentage of fatty acids of fillets of fish fed diets with the different doses of *S. cerevisiae*. 10 fillets were analyzed in each of the three group's replicates. Standard deviations are indicated following average values. Statistical differences among the groups are indicated at level of  $P<0.05$  (\*) or  $P<0.01$  (\*\*)

	Diets		
	Basal Diet (Control)	Diet 1 (added 1‰ <i>S. cerevisiae</i> )	Diet 2 (added 2‰ <i>S. cerevisiae</i> )
C14:0	1.30 $\pm$ 0.12	1.24 $\pm$ 0.15	1.23 $\pm$ 0.17
C14:1	1.25 $\pm$ 0.02	0.23 $\pm$ 0.01	0.21 $\pm$ 0.01
C15:1	0.11 $\pm$ 0.03	1.61 $\pm$ 0.02	2.01 $\pm$ 0.03**
C16:0	19.33 $\pm$ 0.28	19.27 $\pm$ 0.30	19.13 $\pm$ 0.27
C16:1	1.17 $\pm$ 0.12	1.06 $\pm$ 0.11	1.12 $\pm$ 0.11
C17:0	0.34 $\pm$ 0.03	0.29 $\pm$ 0.02	0.31 $\pm$ 0.02
C17:1	0.01 $\pm$ 0.00	0.25 $\pm$ 0.02	0.33 $\pm$ 0.03
C18:0	5.50 $\pm$ 0.15*	4.50 $\pm$ 0.12	4.81 $\pm$ 0.14
C18:1n9c+t	9.83 $\pm$ 0.73	8.50 $\pm$ 0.61	8.48 $\pm$ 0.62
C18:2n6c	6.27 $\pm$ 0.40	5.10 $\pm$ 0.22	4.76 $\pm$ 0.32
C18:3n3	1.50 $\pm$ 0.10	1.53 $\pm$ 0.15	1.31 $\pm$ 0.13
C20:1n9	0.94 $\pm$ 0.20	0.69 $\pm$ 0.15	0.74 $\pm$ 0.18
C20:3n3+ 3n6	1.33 $\pm$ 0.21	1.31 $\pm$ 0.19	1.39 $\pm$ 0.21
C20:4n6	0.72 $\pm$ 0.22	0.54 $\pm$ 0.21	0.49 $\pm$ 0.21
C20:5n3	6.86 $\pm$ 0.35	6.93 $\pm$ 0.36	7.09 $\pm$ 0.38
C22:1n9	0.00 $\pm$ 0.00	0.28 $\pm$ 0.05	0.42 $\pm$ 0.20
C22:6n3	42.24 $\pm$ 1.94	46.08 $\pm$ 2.03	45.43 $\pm$ 1.82
$\Sigma$ SFA	26.47 $\pm$ 0.32	25.29 $\pm$ 0.33	25.48 $\pm$ 0.32
$\Sigma$ MUFA	14.62 $\pm$ 0.72	13.21 $\pm$ 0.61	14.04 $\pm$ 0.62
$\Sigma$ PUFA	58.91 $\pm$ 0.38	61.50 $\pm$ 0.20	60.48 $\pm$ 0.32
DHA/EPA	6.15 $\pm$ 0.05	6.65 $\pm$ 0.07	6.40 $\pm$ 0.05

tilapia diets supplemented with *Echinacea purpurea*, *Origanum dictamnus* and *Saccharomyces cerevisiae*; Peng and Gatlin III (2003) fed hybrid striped bass diet with *Saccharomyces cerevisiae*; Noh *et al.* (1994) fed Israeli carp diets supplemented with antibiotics, enzymes, yeast culture and probiotics; Pooramini *et al.* (2009) fed rainbow trout diet with *Saccharomyces cerevisiae* and Goda *et al.* (2012) fed Nile tilapia with *Saccharomyces cerevisiae* found that increased growth performance and feed utilization efficiency. In contrast, Günter and Jimenez-Montealegre (2004) found that *Bacillus subtilis* added in the feed does not improve growth of tilapia, in fact an adverse effect was reported.

The increased fish growth in the experimental group may possibly be due to many different factors. According to some authors, the beneficial effect of the probiotics were related with improved gut morphology and/or stimulated production of some enzymes on the intestinal tract (Heidarieh *et al.* 2012; De Schrijver and Ollevier, 2000; Lara-Flores *et al.* 2003; Günter and Jimenez-Montealegre, 2004). On the other hand, Barnes *et al.* (2006) reported that some essential and non-essential amino acids in yeast, *Saccharomyces cerevisiae*, were frequently accountable for increasing the palatability of fish food items. Another important factor is that the proteolytic activity of the probiotic bacterium that stimulate and/or produce some enzymes on the intestinal tract (Heidarieh *et al.* 2012). Turbot (*Scophthalmus maximus*), fed with *Vibrio proteolyticus*-based diet, showed enhanced protein digestibility and improved growth. Our study is in line with those results.

No significant differences were observed in crude protein, moisture and ash of muscle in fish fed with different doses of ASC are in line with the outcomes of Kobeisy and Hussein (1995), Oliveira-Teles and Goncalves, (2001) and Fournier *et al.* (2002), who reported that muscle composition was not affected by dietary yeast. However the yeast supplementation significantly affected the fat deposition in the fish body. These results suggest that ASC supplementations act in increasing feed intake with a subsequent enhancement of fat deposition on fish body composition.

Henderson (1996), suggested that monounsaturated fatty acids are preferred for energy production in fish.. The significantly lower fraction of 18:0 in the muscle of fish fed diets with ASC compare to control could possibly be related to higher energy needs for enhanced growth, development of cells and tissues, and concomitant increases in combustion of this fatty acid. Further, it was also determined that the relative content of the important n-3 fatty acids tended to decline as lipid deposition increased in the muscle of fish fed diets with ASC. However, although only minor changes in the fatty acid composition of the trout muscles were identified, the present study did not present any differences in total of SFA, MUFA or PUFA.

In conclusion, the present study designates that ASC positively enhanced growth performance and feed utilization without affecting moisture, protein and ash content, and fatty acid composition, and liver histology of rainbow trout when applied at the dose of 2‰. The ASC could be used as a new probiotic in rainbow trout industry of Turkey.

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