EFFECT OF YEAST SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND RESISTANCE OF GALILEE TILAPIA, *SAROTHERODON GALILAEUS* (L.) TO ENVIRONMENTAL COPPER TOXICITY

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Abstract

The potential use of the dietary probiotics in aqua feeds to enhance growth efficiency and immune response have recently attracted intensive attention. Therefore, this study was conducted to evaluate the growth response of Galilee tilapia, *Sarotherodon galilaeus* (L.) and its resistance to water-born copper toxicity. Fish (1.5 - 2.3 g) were randomly distributed at a rate of 30 fish per 140-L aquarium and fed on diet containing either 0.0 or 10 g yeast/kg diet for 6 weeks after which fish at each treatment were further exposed to either 0.0, 1 ppm Cu for 24 hours, or 5 ppm Cu for 1 hour (T1, T2, or T3 for non-yeast fed groups and T4, T5, or T6 for yeast fed group, respectively). The growth-promoting influence of baker's yeast was observed. Feed utilization was significantly improved when fish fed on a yeast-supplemented diet. Also, yeast supplementation increased serum glucose, lipids, and protein, while no significant effects on creatinine, aspartate amninotransferase (AST), and alanine aminotransferase (ALT). After Cu exposure, serum glucose, total lipids, total protein, creatinine, AST, and ALT increased immediately, while fish of T3 and T6 exhibited higher values than those of T2 and T5. These parameters were lower in fish fed a yeast-supplemented diet than that fed on control diet (T2 vs T5 and T3 vs T6). Fish biochemically recovered the effect of Cu-toxicity in 4-8 days. The Cu residues in fish body in T1 and T4 were approximately the same during the 8-day period, and their values were significantly lower than those of the other experimental groups. The comparison of Cu residue in Cu-treated groups, at the same times, implied that yeast supplementation reduced Cu absorption and accumulation in fish body. Moreover, the Cu residues in T3 and T6 were higher than those of T2 and T5, respectively. The obtained results indicated to the potential use of baker's yeast for Galilee tilapia culture to improve growth performance and reduce the entrance and accumulation of Cu inside fish body.

Keywords: Baker's yeast, *Saccharomyces cerevisiae, Sarotherodon galilaeus*, growth performance, feed utilization, body composition, physiological parameters, Cu toxicity.

INTRODUCTION

Tilapia are widely cultured in the tropical, subtropical, and temperate regions of the world and represent the third largest productive group of farmed finfish species, only after carps and salmonids, with annual growth rate of about 12.2% (El-Sayed, 2006). The most economically important species of tilapia are Nile tilapia, *Oreochromis niloticus,* blue tilapia, *O. aureus,* and Galilaee tilapia, *Sarothrodon galilaeus* (FAO, 2004; El-Sayed, 2006). Nile tilapia and blue tilapia have the highest growth rate, while Galilaee tilapia grows at the slowest rate under warm water temperature, but it has great cold tolerance (Rakocy, 1989). Hence, Galilaee tilapia may be a candidate fish for farming in cold regions and/or their farming may help the farmers to extend the rearing season.

Probiotics are live microbes that may serve as dietary supplements to improve the fish growth (see Gatesoupe, 1999; Irianto and Austin, 2002; Kesarcodi-Watson *et al.*, 2008). Baker's yeast, *Saccharomyces cerevisiae* is a natural product used for the bakers industry that contains various immunostimulating compounds such as β-glucans, nucleic acids, and oligosaccharides, and it has the capability to enhance the growth of various fish species (Oliva-Teles and Goncalves, 2001; Lara-Flores *et al.*, 2003; Abdel-Tawwab *et al.*, 2008). Also, the dietary intake of whole yeast cells has the capability to enhance the immunostimulant properties via enhancing leukocyte phagocytosis, cytotoxicity and respiratory burst (Ortuño *et al.*, 2002; Rodríguez *et al.*, 2003; Cuesta *et al.*, 2004; Esteban *et al.*, 2004; Li and Gatlin, 2005).

The potential use of dietary supplements to enhance environmental tolerance of aquatic animals has increased interest worldwide. For example, Burrells *et al.* (2001 a) found dietary supplementation of nucleotides can enhance growth of Atlantic salmon after being transferred to salt water. Glycine-enriched diet has been shown to significantly enhance survival of oysters after being transferred to freshwater from sea water (Takeuchi, 2007). However, benefits of dietary prebiotic or probiotic supplementation to enhance tolerance to environmental changes have not been reported with any aquatic species to the best our knowledge.

Copper (Cu) sulfate is frequently used as algicide, molluscicide, and herbicide in fish ponds, irrigation and municipal water treatment systems (Boyd, 1990; Tucker and Robinson, 1990; Stoskopf, 1993). The application of copper sulfate in fish ponds is a dose and time dependent. However, the application of high Cu dose for short time may be better than the application of low Cu dose for long time or *vice versa*. In all cases, Cu concentration should not directly affect fish health. Therefore, the present study was conducted to explore the effect of baker's yeast, *S. cerevisiae* used as a

probiotic on the growth performance, feed utilization, and the resistance of Galilaee tilapia, *S. galilaeus* to the further water-born Cu toxicity.

MATERIALS AND METHODS

Diet preparation

Two experimental diets were formulated (30% crude protein and 4.72 kcal/g diet) to contain two levels of baker's yeast (*S. cerevisiae*, B.F.P., Dock Road, Felixstone, UK). The diets contained live baker's yeast as control (0) or 10.0 g/kg diet. The ingredients of each diet were separately blended with additional 100 ml of water per 1 kg diet to make a paste of each diet. The pastes were separately passed through a grinder and pelleted (1 mm diameter) in a paste extruder. The diets were air-dried and stored in plastic bags in a refrigerator (- 2 °C) for further use.

Fish culture and feeding regime

Galilee tilapia, *S. galilaeus* (L.) fry were obtained from fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were kept in an indoor fiberglass tank for 2 weeks for acclimation to the laboratory condition. Chemical analysis at the beginning of the trial was done where a 100-g weight of fish was frozen at – 20 °C. Fish were randomly distributed at a rate of 30 fish (1.5 - 2.3 g) per 140-L aquarium. Each aquarium was supplied with compressed air via air-stones using aquarium air pumps. Settled fish wastes were cleaned daily by siphoning a three-quarter of aquarium's water, which was replaced by aerated water from the storage tank. Water temperature range was 23 - 25 °C. Fish in all treatments were fed to satiation and the feed was offered twice daily at 9:00 and 14:00; five days a week for a period of 6 weeks. Each diet was represented by six replicates. Fish in each aquarium were group-weighed biweekly and dead fish was daily recorded and removed.

Copper exposure trial

After the feeding trial, fish at each yeast level were divided into 3 subgroups, which were further exposed to 0.0 (control), 1 ppm Cu for 24 hours, or 5 ppm Cu for 1 hour (T1, T2, or T3 for non-yeast fed group and T4, T5, or T6 for yeast fed group, respectively). The corresponding weight of $CuSO_4.7H_2O$ was dissolved in 100 ml distilled water, which was distributed in each aquarium's water. Each subgroup was represented by two aquaria (35 fish per each). Three fish from each aquarium were anaesthetized with buffered MS222 (20 mg/L) and blood was collected with a hypodermic syringe from the caudal vein after 0, 1, 2, 4, and 8 days for further physiological assays.

Analysis of water physico-chemistry

Water samples were collected biweekly at 15 cm depth from each aquarium. Dissolved oxygen and temperature were measured on site with a YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Springs, OH, USA). Unionized ammonia was measured using DREL/2 HACH kits (HACH Co., Loveland, CO., USA). The pH degree was measured using a pH-meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). In all treatments, dissolved oxygen concentrations ranged from 5.6 to 5.9 mg/L, pH ranged from 7.6 to 7.9, and unionized ammonia concentration ranged from 0.11 to 0.18 mg/L. All the previous water quality parameters are within the acceptable range for fish growth (Boyd, 1984).

Growth parameters

Growth performance was determined and feed utilization was calculated as described in Abdel-Tawwab *et al.* (2008) as follows:

Weight gain = final weight – initial weight;

Specific growth rate (SGR) = 100 (ln W_2 – ln W_1) / T; where W_1 and W_2 are the initial and final weight, respectively, and T is the number of days in the feeding period;

Feed conversion ratio (FCR) = feed intake / weight gain;

Protein efficiency ratio (PER) = weight gain / protein intake;

Apparent protein utilization (APU; %) = 100 x (protein gain / protein intake) ;

Energy utilization (EU; %) = 100 x (calculated energy gain / calculated energy intake).

Proximate chemical analyses

At the end of growth trial, five fish from each aquarium were collected for the proximate chemical analyses, which were done according to the standard methods of AOAC (1990) for moisture, crude protein, crude lipids, and ash. Moisture content was estimated by drying the samples to constant weight at 85 °C in drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus (Labconco, Labconco corporation, Kansas, Missouri, USA) and crude protein was estimated by multiplying nitrogen content by 6.25. Lipid content was determined by ether extraction in multi-unit extraction Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois, USA) for 16 hours and ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque, Iowa, USA) at 550 °C for 6 hours.

Biochemical measurements

Five fish from each aquarium were anaesthetized with buffered MS222 (30 mg/L) and blood was collected with a hypodermic syringe from the caudal vein. The extracted blood was set without anticoagulant, left to clot at 4 °C, and centrifuged at 5000 rpm for 5 min at room temperature. The collected serum was stored at – 20 °C for further assays. Glucose was determined colorimetrically according to Trinder

(1969). Total protein and total lipids contents was determined colorimetrically according to Henry (1964) and Joseph *et al.* (1972), respectively. Creatinine was measured colorimetrically as described by Henry (1974). Activities of aspartate amninotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically according to Reitman and Frankel (1957).

Metal residue

During Cu exposure trial, three fish were collected from each aquarium after 0, 1, 2, 4, and 8 days were oven-dried at 85 °C until constant weight is reached. A 1.0 g dry weight was ashed in a muffle furnace for 6 hours, then it was digested with concentrated HNO_3 : H_2SO_4 (1:1 v:v) using a muffle furnace, and diluted with 2N HCl to a constant volume. Cu was determined using an atomic absorption spectrophotometer (Thermo 6600, Thermo Electron Corporation, Cambridge, UK).

Statistical analysis

The growth trial data were subjected to one-way ANOVA to evaluate the effect of yeast supplementation. On the other hand, the data obtained during Cu exposure were subjected to two-way ANOVA to evaluate the effect of Cu exposure and the sampling intervals. The differences between means were analyzed at the 5% probability level using Duncan's New Multiple Range test. The software SPSS, version 10 (SPSS, Richmond, Virginia, USA) was used as described by Dytham (1999).

RESULTS

After the 6-week feeding period, fish fed 10.0 g yeast/kg diet exhibited higher final weight, weight gain, and specific growth rate than those fed on the control diet ($P < 0.05$; Table 1). No significant difference ($P > 0.05$) was observed in fish survival due to yeast supplement and its range was 93.3 – 95.6%.

Table 1. Growth performance of tilapia Galilee fed diets containing different levels of live baker's yeast for 6 weeks.

Items	Levels of baker's yeast (g/kg diet)	
	Control (0.0)	10.0
Initial weight (g)	2.86±0.010 a	2.89±0.023 a
Final weight (g)	9.12±0.036 b	12.62±0.064 a
Weight gain (g)	6.26±0.27 b	9.73±0.24 a
Specific growth rate (%/day)	4.142±0.026 b	5.264±0.028 a
Survival rate (%)	93.3±2.2 a	95.6±3.8 a
Feed intake (g feed/fish)	8.49±0.075 b	9.85±0.098 a
Feed conversion ratio	1.36±0.137 b	1.01±0.109 a
Protein efficiency ratio	2.63±0.051 b	4.09±0.068 a
Apparent protein utilization (%)	41.18±0.695 b	57.97±0.989 a
Energy utilization (%)	29.25±0.485 b	34.74±0.581 a

The same letter in the same row is not significantly different at $P < 0.05$.

Moreover, feed intake was significantly highest, while FCR was significantly lower when fish fed yeast-supplemented as compared the control group ($P < 0.05$; Table 2). Also, PER, APU, and EU were significantly enhanced when yeast was included in fish diet ($P < 0.05$).

Table 2. Proximate chemical analysis (%; on dry matter basis) of whole body of Galilee *tilapia* fed diets containing different levels of live baker's yeast.

Items (%)		Levels of baker's yeast (g/kg diet)	
	Initial	Control (0.0)	10.0
Moisture	73.2±0.75	73.9±0.95 a	74.5±1.22 a
Crude protein	53.7±0.91	58.5±1.34 b	62.4±1.83 a
Total lipids	23.6±0.73	28.4±1.64 a	22.3±2.18 b
Ash	21.3±0.44	10.9±0.56 b	13.3±1.36 a

The same letter in the same row is not significantly different at $P < 0.05$.

On the other hand, fish fed the diet supplemented with yeast had significantly ($P < 0.05$) higher whole-body contents of protein and ash, and lower lipid content than fish fed the basal control diet (Table 3).

Prior to the Cu exposure, serum glucose, total protein, and total lipids of fish fed yeast-supplemented diet were significantly higher than those fed the control diet

(Table 3). No significant differences in serum creatinine, AST, and ALT levels were observed between the different treatments ($P > 0.05$).

Table 3. Biochemical changes of Galilee tilapia fed diets containing different levels of live baker's yeast.

Items (%)	Levels of baker's yeast (g/kg diet)	
	Control (0.0)	10.0
Glucose (g/L)	0.614±0.0082 b	0.718±0.0043 a
Total protein (g/L)	22.4±0.49 b	25.1±0.65 a
Total lipids (g/L)	31.8±0.84 b	35.8±0.22 a
Creatinine (mg/L)	8.4±0.19 a	8.1±0.17 a
Aspartate amninotransferase (IU/L)	11.0±0.58 a	10.7±0.88 a
Alanine aminotransferase (IU/L)	18.7±0.88 a	19.3±0.88 a

The same letter in the same row is not significantly different at $P < 0.05$.

When fish were exposed to water-born Cu, blood parameters were significantly affected by Cu treatment and yeast supplementation ($P < 0.05$), meanwhile no significant changes were observed in T1 and T4 ($P > 0.05$; Figs 1 and 2). Serum glucose, total lipids, and total protein increased immediately when fish were exposed to Cu, irrespective to yeast supplement. Fish in T3 and T6 exhibited higher serum glucose, lipids, and protein than that those of T2 and T5 ($P < 0.05$; Fig 1). On the other hand, the levels of these parameters were lower in fish fed a yeast-supplemented diet than that fed on control diet (T2 vs T5 and T3 vs T6; $P < 0.05$).

Fish recovered from the effect of Cu-toxicity after 2 - 8 days, however, no significant differences in serum glucose and total lipids were observed among different treatments. On the other hand, total serum protein varied dramatically ($P < 0.05$) among different treatments (Fig.1). In Cu-exposed treatments, total serum protein needed more time to recover, and it was close to the control group in the 8th day. Similarly, levels of serum creatinine, AST, and ALT increased significantly after Cu exposure and declined by time to be close to that of the control group in the 8th day (Fig 2). On the other hand, fish fed yeast-supplemented diet exhibited lower values of creatinine, AST, and ALT than those fed on the control diet (T2 vs T5 and T3 vs T6; $P > 0.05$).

The Cu residues in the control groups (T1 and T4) were approximately the same during the 8-day period ($P > 0.05$), and their values were significantly lower than those of the other experimental groups ($P < 0.05$; Fig 3). Moreover, the maximum Cu residue in T2 and T5 was obtained at the second day, whereas it was obtained at the first day in T3 and T6. In Cu-exposed groups, Cu concentrations declined by time and the lowest levels were obtained after the 8th day. It is also noticed that yeast supplement groups (T5 and T6) absorbed less Cu than the other groups (T2 and T3; Fig 3).

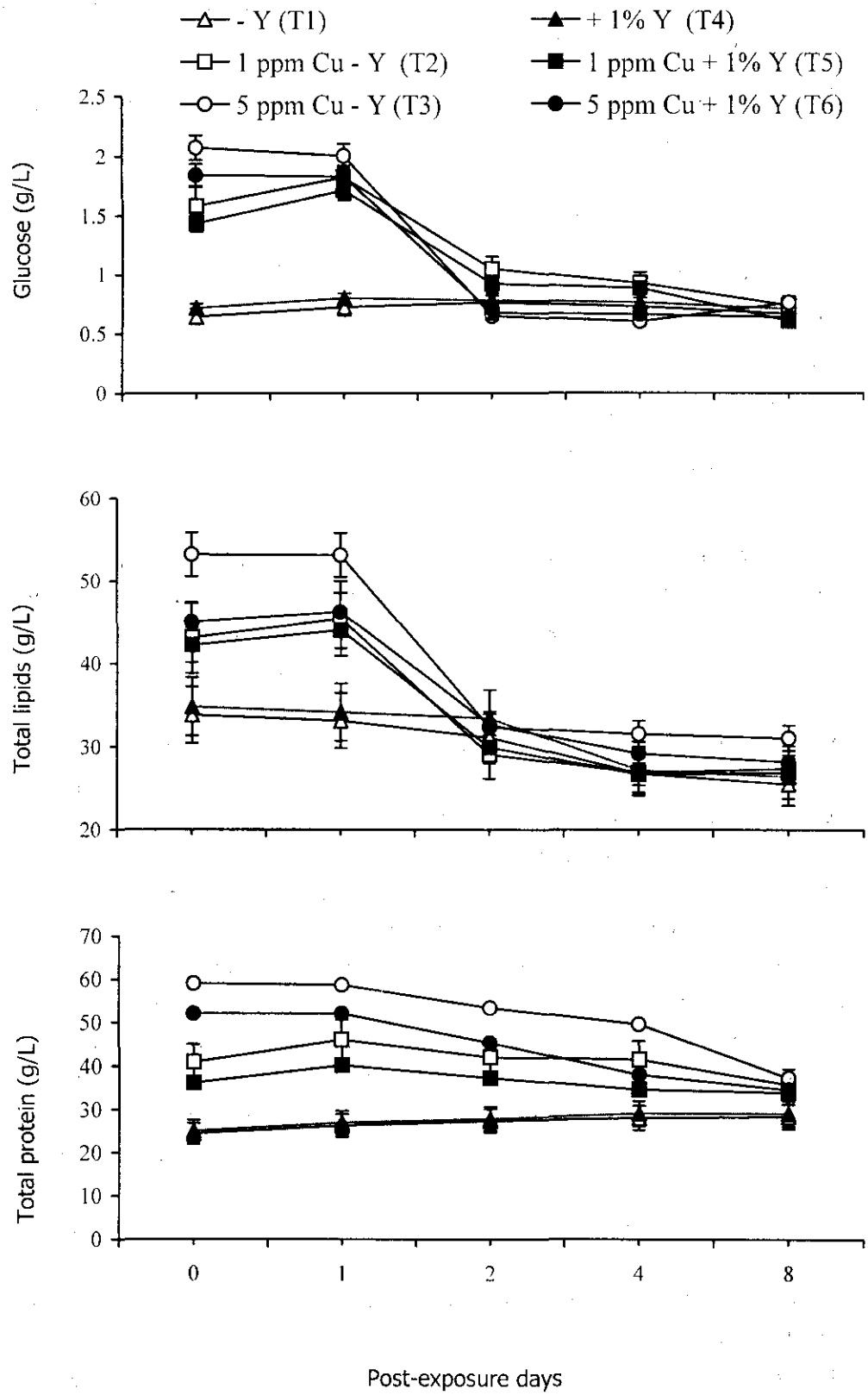

Figure 1. Changes in serum glucose, lipid, and protein of Galilee tilapia fed different levels of live baker's yeast for 6 weeks and post-exposed to different Cu levels.

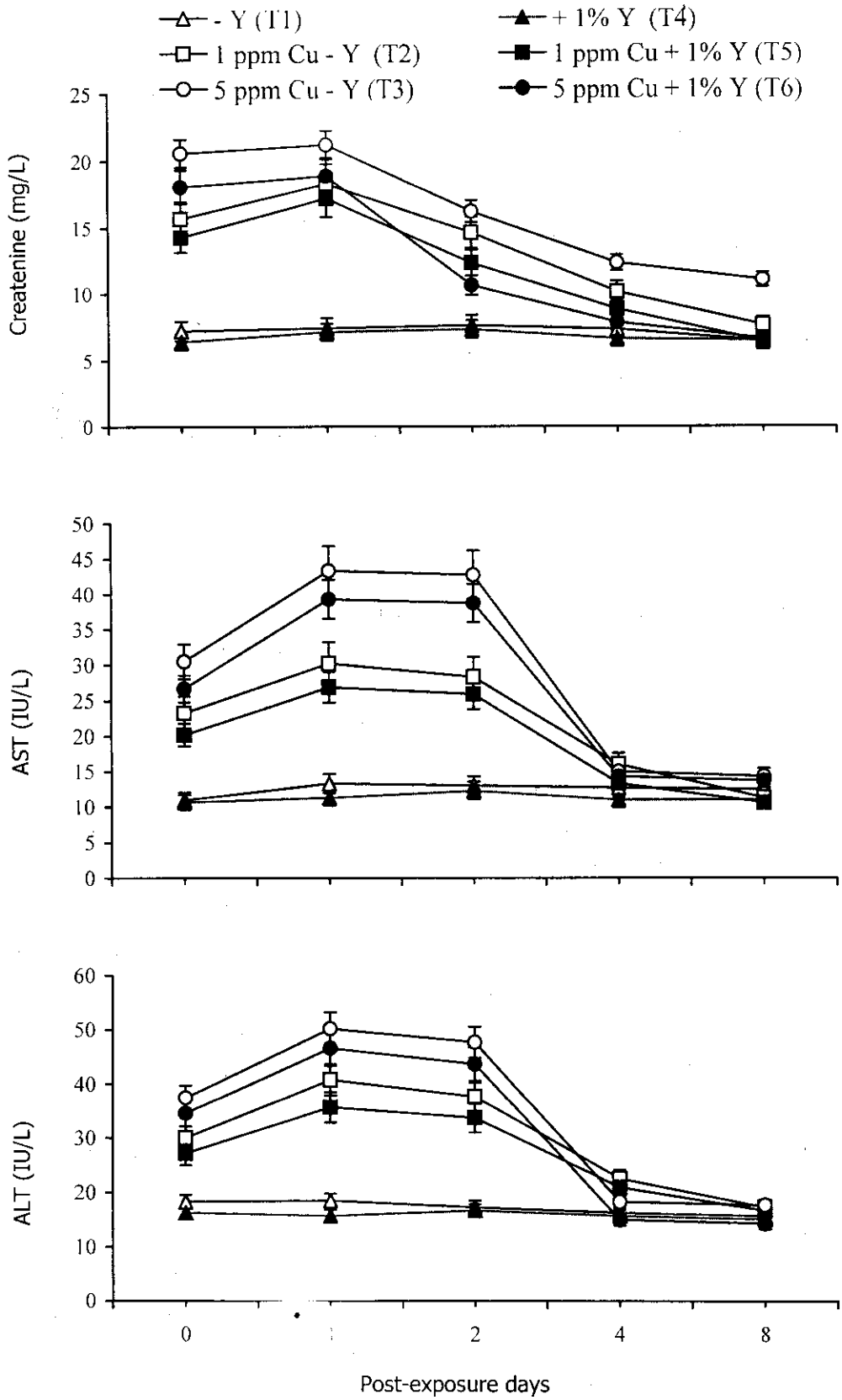

Figure 2. Changes in serum creatinine, AST, and ALT of Galilee tilapia fed different levels of live baker's yeast for 6 weeks and post-exposed to different Cu levels.

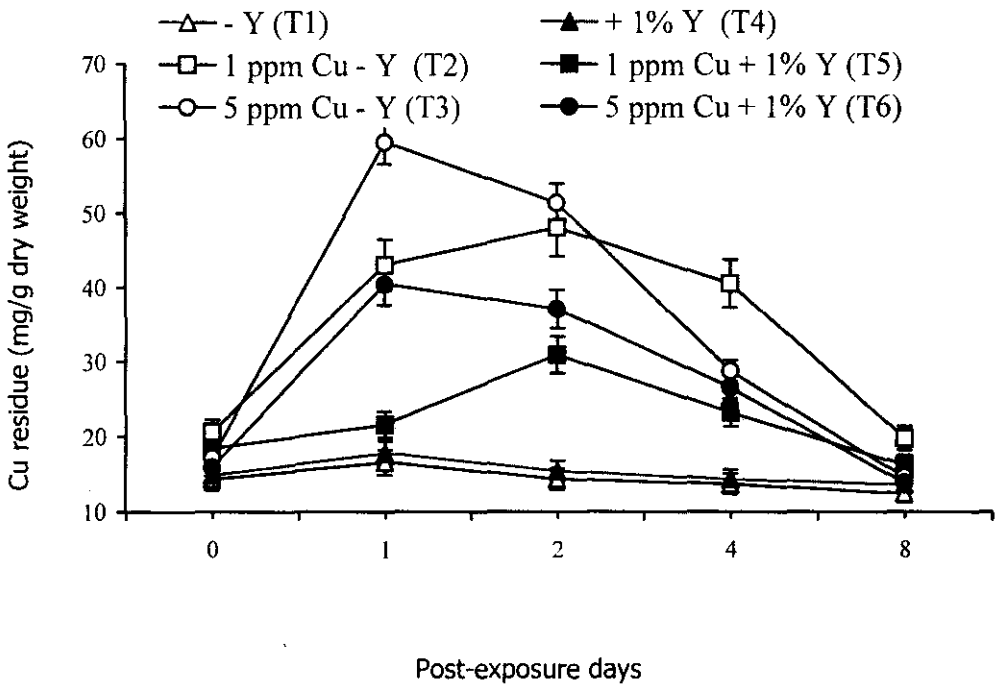

Figure 3. Changes in Cu residue (mg/g dry weight) of Galilee tilapia fed different levels of live baker's yeast for 6 weeks and post-exposed to different Cu levels.

DISCUSSION

Prepared diets provide not only the essential nutrients that are required for normal growth and physiological functions but also may serve as a medium by which fish receive certain compounds that may alter endocrine activity, immunity and other physiological responses (Gatlin III, 2002). The dietary supplementation of live baker's yeast, *S. cerevisiae* evaluated in the present study resulted in an enhancement of fish growth and feed utilization. These results agreed with studies involving other fish species, in which enhanced growth occasionally has been associated with dietary supplementation of brewers yeast, *S. cerevisiae* (Lara-Flores *et al.*, 2003; Li and Gatlin III, 2005; Abdel-Tawwab *et al.*, 2008). The improved fish growth and feed utilization may possibly be due to the acceleration of the digestive system maturation and the increased nutrient digestibility (Tovar-Ramírez *et al.*, 2004; Waché *et al.*, 2006). Moreover, Buts *et al.* (1994) reported that yeast may release spermine and spermidine in the digestive tract, which playing a fundamental role in proliferating, fast growing, and regenerating tissues (Peulen *et al.*, 2002). It is therefore possible that spermine and spermidine production by yeasts may explain at least partly the effect observed on fish growth and feed utilization.

The better feed utilization with yeast supplementation may have been because yeast might play a role in enhancing feed intake resulting in higher fish growth, due to

the high feed intake, protein utilization, energy utilization, and the high nutrient digestibility. On the other hand, changes in protein and lipids contents in fish body could be linked with changes in their synthesis and/or deposition rate in muscle (Smith, 1981; Fauconneau, 1984; Soivio *et al.*, 1989; Abdel-Tawwab *et al.*, 2006).

Biochemical analyses often provide vital information helping in health assessment and management of cultured fish (Pincus, 1996; Cnaani *et al.*, 2004; Řehulka *et al.*, 2004). In the present study, fish fed on a diet containing 10.0 g yeast/kg diet exhibited higher glucose, protein, and lipids, meanwhile the levels of creatinine, AST, and ALT were not affected. In this regard, Taoka *et al.* (2006) found that diet containing commercial probiotic increased the plasma protein of Japanese flounder, *Paralichthys olivaceus*. In addition, Carver and Walker (1995) and Sato *et al.* (1995) reported that dietary yeast could influence the levels of various lipids and/or fatty acids in certain tissues, such as erythrocytes, plasma, liver, or brain. On the other hand, the non-significant differences in creatinine, AST, and ALT suggest that yeast supplementation had no toxic effects.

The biochemical parameters and Cu residues were significantly affected by Cu dose and recovery time, where these parameters were higher in T3 vs T2 and T6 vs T5 ($P < 0.05$; Figs 1 and 2). Moreover, The Cu content in fish body after Cu exposure treatments was significantly higher than those in the control group. These results are more expected and the response of Galilaee tilapia against Cu in the present study was similar to that obtained with common carp (Peyghan *et al.*, 2003), Nile tilapia (Abdel-Tawwab *et al.*, 2007a), and African catfish (Abdel-Tawwab *et al.*, 2007b). The Cu residues in fish bodies exposed to Cu were declined after the 2nd day may be due to the gradual Cu excretion through the biliary system and gut. The ability of fish to respond rapidly to Cu and reestablish their physiological control (homeostasis) has been previously shown in rainbow trout (Lauren and McDonald 1987; Grosell *et al.* 1997) and common carp (Peyghan *et al.*, 2003).

On the other hand, fish fed on yeast supplement could tolerate Cu toxicity irrespective to its concentration. These results suggested that yeast supplements may increase the immuno-stimulants that may have played a role in reducing Cu toxicity. Taoka *et al.* (2006) found that probiotic supplement increased the excretion of fish skin mucus, which has an important defense role in reducing the entrance of Cu into fish blood. In similar regards, Burrells *et al.* (2001 b) reported that dietary nucleotides (derived from yeast) increased the resistance of salmonids to some parasites such as sea lice. Li *et al.* (2005) reported that the dietary nucleotides supplements could potentially be effective in reducing mortality of juvenile red drum (*Sciaenops ocellatus*) and the virulence of amyloodiniosis in conjunction with multiple chemical exposures

and environmental control including hydrogen peroxide and copper sulfate treatment and/or temperature and salinity adjustments. Taoka *et al.* (2006) found that Japanese flounder, *P. olivaceus* fed probiotic could tolerate exposure-to-air and heat stresses and *Vibrio anguillarum* resulted in significantly higher survival in the probiotics-treated groups than the control group.

From the present study, 10 g baker's yeast/kg diet is recommended to surpulenent practical diets of Galilaee tilapia to improve fish growth and feed utilization, and resist the environmental Cu toxicity that may be occurred in aquatic system.

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