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Effects of dietary baker's yeast extract on the growth, blood indices and histology of Nile tilapia (*Oreochromis niloticus* L.) fingerlings

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Abstract

Nile tilapia, Oreochromis *niloticus* (average initial weight, 5.91 ± 0.04 g), were fed four isonitrogenous and isolipidic diets for 84 days. The diets contained four levels of yeast extract (CW-I) rich in nucleotides and β-glucan: 0 (control), 5, 10 and 15 g/kg diet. Weight gain increased linearly, whereas the feed conversion ratio decreased linearly with increasing levels of yeast extract. The diet containing 15 g/kg yeast extract resulted in significantly better ($p < 0.05$) specific growth rates and protein efficiency ratios. No significant ($p > 0.05$) differences were found in dry matter, protein, lipid or ash contents or in haematocrit, haemoglobin or total protein contents among the treatment groups. Blood sample profiles showed an increase in white and red blood cells in fish fed 15 g/kg yeast extract in comparison with the other treatment groups. The fish fed the diets with 10 and 15 g/kg yeast extract had significantly higher albumin and globulin levels than the control group, whereas decreased levels of cholesterol and triglycerides, aspartate aminotransferase and alanine aminotransferase were noted in fish fed the diet with 15 g/kg yeast extract. Histological analysis of the liver and intestine in fish fed the control diet showed a slightly abnormal structure in these organs. Only in fish fed diets supplemented with the highest amount of yeast extract was the structure of the hepatocytes and villi almost unchanged, which indicated that the yeast nucleotides could improve hepatic function and promote liver and gut restoration.

KEYWORDS
growth, haematology, histology, nucleotides, *Oreochromis niloticus*, yeast extract

1 | INTRODUCTION

One of the main challenges in achieving productive, feasible, sustainable aquaculture is to develop alternative prophylactics that could help to maintain high animal welfare standards that foster better production and higher profits. Fish diets should not only provide the essential nutrients that are required for normal physiological functioning, but also serve as the medium by which fish receive other components that affect their health (Goda et al., 2018; Li & Gatlin, 2004). Baker's yeast, or *Saccharomyces cerevisiae* yeast, is a particularly important natural bioproduct as it contains immune-stimulating compounds such as nucleotides, β-glucan, mannan oligosaccharides and chitin, and it has been proved to influence the fish immune response and to promote growth (Abdel-Tawwab, 2012). On the other hand, commercial brewer's yeast is inactive yeast (dead yeast cells) that is a by-product of brewing. The cell wall, which can comprise 200—250 g/kg of the dry weight of the cell, consists of about 85%–90% polysaccharide. The polysaccharide

component consists of a mixture of mannan, glucan and small amounts of chitin (Nguyen, Fleet, & Rogers, 1998). Numerous studies have focused on the effect of mannan oligosaccharides, glucans and chitin on the immune response in different fish species and indicate that these compounds strongly stimulate fish immune systems (Couso, Castro, Magariños, Obach, & Lamas, 2003; Torrecillas et al., 2007). Yeast extract is the product of the enzymatic digestion of the yeast cell constituents by endogenous and exogenous yeast enzymes (Bekatorou, Psarianos, & Koutinas, 2006). Yeast extract is considered an important source of nucleotides in the form of nucleic acids (Ferreira, Pinho, Vieira, & Tavarela, 2010). Nucleotides are low molecular weight biological compounds that play important roles in essential physiological and biochemical functions (Carver & Walker, 1995). Nucleotides are synthesized de novo in most tissues, but some immune and intestinal cells lack or cannot execute this process and depend on exogenous dietary supply (Quan, 1992). Hence, the administration of pure nucleotides guarantees increased availability to the body at times of high demand for various physiological activities (Biswas et al., 2012). High dietary concentrations of nucleotides can also compromise growth and protein accretion (Oliva-Teles, Guedes, Vachot, & Kaushik, 2006; Peres & Oliva-Teles, 2003). It has been demonstrated that nucleotides added to basal diets can affect positively fish growth (Li et al., 2005), innate and adaptive immune responses (Li & Gatlin, 2004; Li, Lewis, & Gatlin, 2004; Sakai, Taniguchi, Mamoto, Ogawa, & Tabata, 2001) and disease resistance (Barros et al., 2013). However, most experiments on the effect of baker's yeast on the growth and physiological condition in different fish species have focused on investigating the effects of whole yeast cells, or of bioactive components that were isolated from whole yeast cells, such as nucleotides or β-glucan. To the best of the authors' knowledge, there is little information regarding the effect on fish immune responses of baker's yeast extract that contains both nucleotides and β-glucan. Therefore, this study was designed to evaluate the efficacy of baker's yeast extract (CW-I) supplementation on the growth, feed utilization, haematological, and histological and biochemical blood parameters of Nile tilapia, *Oreochromis niloticus* L.

2 | MATERIALS AND METHODS

2.1 | Experimental design and culture technique

Nile tilapia (*O. niloticus*) fingerlings were collected from the Fish Research Station, El-Kanater El-Khayria, National Institute of Oceanography and Fisheries, Cairo, Egypt, and held for 2 weeks in indoor fibreglass tanks for acclimation. Prior to the beginning of the experiment, the fish were acclimatized to experimental conditions and manually fed a commercial diet (300 g/kg crude protein) twice daily to apparent satiation for 7 days. After acclimatization, 600 Nile tilapia fingerlings with an average initial body weight of 5.91 ± 0.04 g were stocked into 12 concrete ponds (0.5 m^3). Each pond was stocked with 100 fish and maintained in freshwater at 26°C (±2.0) under a natural photoperiod. All dietary treatments were tested in triplicate, and each pond was considered to be an experimental unit. During the experiment, the fish were fed manually two times daily to apparent satiation at 09:00 and 15:00. The total fish weight in each pond was determined every 2 weeks to check their growth. Feeding was stopped 24 hr prior to weighing. A volume of 30% of the freshwater in each pond was renewed through the outlet at the bottom of the ponds daily before feeding. They were provided with continuous aeration to maintain the dissolved oxygen level near saturation, and the fish were held under natural light. Water temperature and dissolved oxygen were measured every other day using a YSI 58 oxygen meter (YSI Company, Yellow Springs Instrument, Yellow Springs, Ohio, USA). Total ammonia and nitrite were measured twice weekly with a DREL 2000 spectrophotometer (Hash Company, Loveland, CO, USA). Total alkalinity and chloride were monitored twice weekly by titration; pH was monitored twice weekly using a pH meter (Orion pH meter, Abilene, Texas, USA). All tested water quality criteria (temperature, DO, pH, total ammonia and nitrite) were estimated according to standard methods as described elsewhere (Hassaan, Goda, Mahmoud, & Tayel, 2014).

2.2 | Experimental diets

Four isonitrogenous and isolipidic diets were formulated (Table 1). Soybean meal contributed the major portion of dietary protein. The proximate composition of the experimental diets was within the desired formulated values with about 300 g/kg crude protein and 19.45 MJ/kg gross energy. The control diet contained no added yeast extract. Three diets were supplemented with 5, 10 and 15 g/kg yeast extract per diet, respectively (Mark Co., Ltd., Tokyo, Japan [CW-I]). The final product was in the form of a fine powder, containing nucleotides (104.4 g/kg) and β-glucan (70.3 g/kg). The ingredients were ground into fine powder through 200 μm mesh. The quantities of extract were mixed with 6 ml of distilled water and added to the base ingredient, all the ingredients were thoroughly mixed with soybean oil, and then, the mixture was passed through a laboratory pellet mill (2-mm die; California Pellet Mill, San Francisco, CA, USA) at the National Institute of Oceanography and Fisheries, Cairo Governorate, Egypt, and the temperature of pellets in this stage did not exceed 40°C. Diets were dried in open air and then packed in cellophane bags and stored at −20°C until used.

2.3 | Growth parameters

At the end of the feeding trial, 24 hr following the last feeding, all the fish were counted and weighed to determine final body weight (g), weight gain (WG), specific growth rate (SGR, % per day), feed conversion ratio (FCR), protein efficiency ratio (PER) and feed intake. The growth response parameters were calculated as follows:

Weight gain (WG) = final body weight (g) − initial body weight (g)

Specific growth rate (SGR) = 100 × (Ln $W2$ − Ln $W1$)/T

TABLE 1 Composition and proximate analysis of the basal diet (g/kg dry matter)

Ingredients	Control	5 g/kg yeast extract	10 g/kg yeast extract	15 g/kg yeast extract
Fish meal	100	100	100	100
Soybean meal	460	460	460	460
Yellow corn	295	295	295	295
Wheat bran	100	95	90	85
Soybean oil	30	30	30	30
Vitamins and minerals[a]	15	15	15	15
Yeast extract (CW-I)	0	5	10	15
Proximate analysis (g/kg dry matter basis)				
Crude protein	300.50	298.80	298.00	297.30
Lipids	56.91	57.20	56.71	57.21
Ash	54.30	54.12	53.81	53.21
Total carbohydrate[b]	5,883	589.88	591.84	592.28
Gross energy (MJ/kg)[c]	19.45	19.44	19.42	19.43

Notes. [a]Vitamin and mineral mix: $MnSO_4$, 40 mg; MgO, 10 mg; K_2SO_4, 40 mg; $ZnCO_3$, 60 mg; KI, 0.4 mg; $CuSO_4$, 12 mg; ferric citrate, 250 mg; Na_2SeO_3, 0.24 mg; Co, 0.2 mg; retinol, 40,000 IU; cholecalciferol, 4,000 IU; α-tocopherolacetate, 400 mg; menadione, 12 mg; thiamine, 30 mg; riboflavin, 40 mg; pyridoxine, 30 mg; cyanocobalamin, 80 mcg; nicotinic acid, 300 mg; folic acid, 10 mg; biotin, 3 mg; pantothenic acid, 100 mg; inositol, 500 mg; and ascorbic acid, 500 mg. [b]Total carbohydrate = 100 − (crude protein + lipid + ash). [c]Calculated using gross calorific values of 23.63, 39.52 and 17.15 kJ/g for protein, fat and carbohydrate, respectively, according to Brett (1973).

where Ln = natural log; *W*1 = initial body weight; *W*2 = final body weight; and *T* = study period (84 days). Feed conversion ratio (FCR) = Feed intake (g)/WG (g). Protein efficiency ratio (PER) = WG (g)/Protein intake (g).

2.4 | Haematological and biochemical blood analysis

At the end of the experimental trial, ten fish were collected randomly from each of the treatment and control groups. The fish were anaesthetized with benzocaine (50 mg/L; Sigma-Aldrich) before blood was drawn. Blood samples were collected from the caudal vein of the fish from all treatments and were divided into two portions. The first portion was collected with anticoagulant 10% EDTA (ethylenediaminetetraacetate) to measure haematocrit (Htc), haemoglobin (Hb), red blood cells (RBCs) and white blood cells (WBCs). Htc was determined as described by Reitman and Frankel (1957), and haemoglobin (Hb) was determined with haemoglobin kits, which is the standard procedure for the cyanmethemoglobin method. RBCs were counted under a light microscope using a Neubauer hemocytometer after blood dilution with phosphate-buffered saline (pH 7.2), and the WBCs were determined according to Barros, Ranzani-Paiva, Pezzato, Falcon, and Guimaraes (2009). The second portion of the blood sample was allowed to clot overnight at 4°C, and then, it was centrifuged at 3,000 g for 10 min. Nonhaemolysed serum was collected and stored at −20°C until analysis. The levels of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured according to the method described by Reitman and Frankel (1957), whereas serum creatinine was measured with the colorimetric method and enzymatic determination method described by Henary, Cannon, and Winkleman (1974). In addition, serum total protein, albumin and globulin were determined spectrophotometrically using the methods described by Doumas et al. (1981).

2.5 | Histological analysis

On Day 84 of the experiment, the livers and digestive tract midsections of five fish from each treatment were excised carefully and fixed in 10% formalin, dehydrated in ascending grades of alcohol and cleared in xylene. The fixed tissues were embedded in paraffin wax, and 5-μm sections were cut with a Euromex Holland microtome (Arnhem, The Netherlands). The sections were stained with the Harris haematoxylin and eosin (H&E) method. Next, these sections were examined microscopically, and photographs were taken with a microscope camera (Bernet, Schmidt, Meier, Burkhardt-Holm, & Wahli, 1999).

2.6 | Chemical composition

At termination of the trial, a random sample of five individual fish were sampled from each pond, oven-dried at 105°C for 24 hr, ground and stored at −20°C for subsequent analysis. Proximate analysis was conducted on both diet and fish samples. Dry matter, total lipids, crude protein and ash contents were all determined with standard methods (AOAC 1995). Dry matter was determined after drying the samples in an oven (105°C) for 24 hr. Ash was determined by incineration at 550°C for 12 hr (AOAC 1995; according

to method number 942.05). Crude protein was determined with the micro-Kjeldahl method, $N \times 6.25$ (using a Kjeltec 1030 Auto Analyzer; Tecator, Höganäs, Sweden; according to method number 984.13), and crude fat was determined by Soxhlet extraction with diethyl ether (40–60°C) (AOAC 1995), according to method number 920.39). Total carbohydrate was computed by subtracting the sum of the crude protein, crude lipid and ash contents from 100.

2.7 | Statistical analysis

Data were analysed statistically with ANOVA using the SAS ANOVA procedure (Statistical Analysis System 2004). The data were submitted to one-way classification variance analysis. Duncan's multiple range test was used to compare differences among treatment means when significant *F* values were obtained (Duncan 1955) at a level of significance of $p < 0.05$. A linear model was performed with SigmaPlot version 8 (SPSS Inc., Chicago, IL, USA) for the response variable using means ± *SE*. All percentage data were arcsine-transformed prior to analysis (Zar, 1984); however, the data are presented untransformed to facilitate comparisons.

3 | RESULTS

3.1 | Growth parameters

The positive water quality criteria were associated with good growth performance as there were no mortalities in any of the treatments throughout the experiment. The growth performance of *O. niloticus* fed the experimental diets is presented in Table 2. There were no significant differences in initial weights among the treatment groups; however, after 84 days, the group fed the diet containing 15 g/kg yeast extract had the highest final body weights and specific growth rates (SGR). Figure 1 shows that weight gain (WG) increased linearly as dietary supplementation increased. Feed intake in this study increased significantly with increased levels of yeast extract. Figure 1 shows that the feed conversion ratio (FCR) decreased linearly as dietary supplementation increased. The addition of yeast extract to the feed also produced a better protein efficiency ratio (PER) with values significantly ($p < 0.05$) higher than those in the diet unsupplemented with yeast extract (control), more specifically in the groups treated with 15 g/kg yeast extract. There was no significant difference in the final body weight, SGR or PER between the groups fed diets with 5 and 10 g/kg yeast extract.

3.2 | Chemical composition of whole fish

According to the body analysis composition data at the end of the experiment, supplementing the feed with yeast extract did not have a significant ($p > 0.05$) impact on dry matter, lipid, crude protein or ash contents of the fish (Table 3).

3.3 | Haematological parameters

Table 4 shows the effect of yeast extract on Nile tilapia haematological indices including haematocrit (Htc), haemoglobin (Hb) and the red blood cell (RBC) and white blood cell (WBC) counts. No significant differences were noted in Htc or Hb levels among all the treatments. RBC and WBC counts were significantly ($p < 0.05$) higher in the fish fed the highest level of yeast extract (15 g/kg diet) in comparison with other treatment groups.

3.4 | Biochemical blood parameters

According to the results of the analysis, the fish that received the highest concentration of yeast extract (15 g) in their diets exhibited significantly ($p < 0.05$) lower AST and ALT activity in comparison with the values noted in the other treatments (Table 5). No significant ($p > 0.05$) differences were noted in the total protein levels in any of the treatments. Fish fed diets containing 10 and 15 g/kg yeast extract had significantly higher albumin and globulin levels than did the fish fed the control diet (Table 5). Some of the other recorded parameters, such as cholesterol and triglyceride levels, in the fish supplemented with yeast extract were significantly lower ($p < 0.05$) than those in the control group. The lowest cholesterol and triglyceride levels were recorded in fish fed diets with 15 g/kg yeast extract (Table 5).

3.5 | Histology

The liver and intestine histology of Nile tilapia fed diets with different levels of yeast extract is illustrated in Figure 2. The histological changes in fish liver and intestines were assessed with light microscopy, which revealed that the fish fed the control diet exhibited

TABLE 2 Growth indices and nutrient utilization of *Oreochromis niloticus* 84 days after feeding of yeast extract-supplemented diets

Items	Control	5 g/kg yeast extract	10 g/kg yeast extract	15 g/kg yeast extract	*p* value
Initial body weight (g/fish)	5.89 ± 0.56	5.84 ± 0.49	5.84 ± 0.54	5.87 ± 0.45	0.875
Final body weight (g/fish)	34.40 ± 1.56^{c}	40.12 ± 1.71^{b}	40.45 ± 1.15^{b}	42.99 ± 1.78^{a}	0.014
Specific growth rate (% per day)	1.95 ± 0.12^{c}	2.12 ± 0.11^{b}	2.13 ± 0.09^{b}	2.31 ± 0.13^{a}	0.012
Feed intake (g/fish)	52.88 ± 1.13^{c}	54.78 ± 1.88^{b}	55.39 ± 1.90^{b}	56.33 ± 1.18^{a}	0.023
Protein efficiency ratio	1.82 ± 013^{c}	2.12 ± 011^{b}	2.12 ± 013^{b}	2.23 ± 0.12^{a}	0.017

Note. Results are presented as means ± *SE* of triplicate observations. Means in the same row with different superscript letters are significantly different ($p < 0.05$).

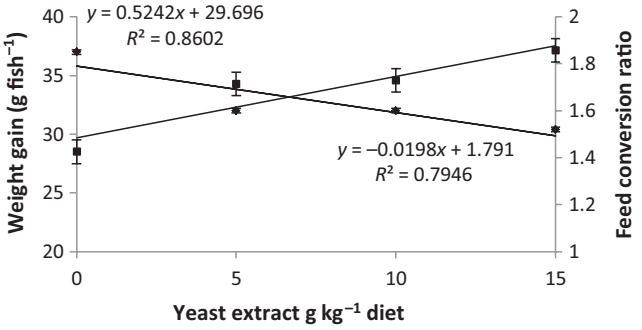

FIGURE 1 Effect of dietary yeast extract on weight gain and feed conversion ratio of *Oreochromis niloticus* ($p < 0.05$). Data are means ± *SE*

slight changes in these organs. Changes in the liver included degeneration and congestion in the blood sinusoids of fish fed the diet unsupplemented with yeast extract (control; Figure 2a). Also, the intestine showed degeneration in mucosa and necrosis in submucosal layers in fish fed the diet unsupplemented with yeast extract (control; Figure 2c). However, fish fed the diet supplemented with 10 or 15 g/kg exhibited an almost normal hepatocyte structure (Figure 2b) and intestinal layers (Figure 2d).

4 | DISCUSSION

The diet supplemented with high levels of yeast extract (15 g/kg) increased the growth rate and feed utilization of Nile tilapia. The yeast extract used in the present study contained nucleotides (10.44 g/kg) and β-glucan (70.3 g/kg), which facilitated fish growth (Carver, 1994). Supplemented diets with 0.1% β-glucan improved Nile tilapia weight gain (Welker, Lim, Yildirim-Aksoy, & Klesius, 2012). Diets containing β-glucan and mannan oligosaccharides (MOS) have also previously been found to improve the growth performance of Nile tilapia and beluga, *Huso huso* (Selim & Reda, 2015; Ta'ati, Soltani, Bahmani, & Zamini, 2011). In our experiment, feed intake increased significantly with increasing levels of *S. cerevisiae* extract in the diet. This could have been because the extract nucleotide contents of adenosine monophosphate, inosine monophosphate, uridine monophosphate and adenosine diphosphate are proven to be palatability enhancers and feed attractants (Li & Gatlin, 2006; Oliva-Teles et al., 2006). Furthermore, dietary nucleotide supplementation has also been shown to enhance growth in other fish species such as Atlantic salmon (Burrells, Williams, & Forno, 2001); grouper, *Epinephelus malabaricus* (Lin, Wang, & Shiau, 2009); and rainbow trout, *Oncorhynchus mykiss* (Tahmasebi-Kohyani, Keyvanshokooh, Nematollahi, Mahmoudi, & Pasha-Zanoosi, 2011). Goda, Mabrouk, Wafa, and El-Afifi (2012) indicated that feed conversion ratio, dietary protein and energy utilization in diets supplemented with *S. cerevisiae* were higher than in the diet unsupplemented with *S. cerevisiae*.

However, Jarmołowicz et al. (2012) reported that supplementing diets with yeast extract (NuPro®) did not significantly impact the growth rates of juvenile European pikeperch, *Sander lucioperca*. The reasons for the differences among these studies could stem from the differences in species, physiological conditions and the type of basal ingredients in the diets.

No significant ($p > 0.05$) differences were noted in the analysis of the proximate composition of Nile tilapia fed the experimental diets. Jarmołowicz et al. (2012) observed that brewer's yeast extract supplementation did not interfere with the metabolism or deposition of nutrients in juvenile pikeperch tissues. The present data were confirmed by the observations of Peres and Oliva-Teles

TABLE 3 Chemical composition of *Oreochromis niloticus* 84 days after feeding of yeast extract-supplemented diets (g/kg wet basis)

Items	Control	5 g/kg yeast extract	10 g/kg yeast extract	15 g/kg yeast extract	*p* value
Dry matter	272.17 ± 1.89	277.02 ± 2.01	278.34 ± 1.87	279.46 ± 2.10	0.084
Crude protein	158.25 ± 1.23	159.21 ± 1.52	159.98 ± 2.11	162.02 ± 1.15	0.781
Lipid	63.23 ± 1.00	65.89 ± 1.17	65.23 ± 1.12	67.24 ± 1.12	0.332
Ash	31.22 ± 1.12	31.98 ± 0.98	32.10 ± 0.90	33.02 ± 0.91	0.416

Note. Results are presented as means ± *SE* of triplicate observations. Means in the same row with different superscript letters are significantly different ($p < 0.05$).

TABLE 4 Haematological parameters of *Oreochromis niloticus* 84 days after feeding of yeast extract-supplemented diets

Items	Control	5 g/kg yeast extract	10 g/kg yeast extract	15 g/kg yeast extract	*p* value
Haematocrit (%)	14.57 ± 0.89	14.27 ± 0.99	14.68 ± 0.79	14.80 ± 0.78	0.780
Haemoglobin (g/dl)	10.31 ± 0.65	10.45 ± 0.50	10.65 ± 0.52	11.00 ± 0.46	0.452
WBCs ($\times 10^{-3}$ mm^{-3})	35.67 ± 0.95^{d}	37.00 ± 0.87^{c}	38.33 ± 1.01^{b}	40.00 ± 1.00^{a}	0.018
RBCs ($\times 10^{-3}$ mm^{-3})	1.81 ± 0.26^{b}	1.83 ± 0.26^{b}	1.85 ± 0.16^{b}	1.91 ± 0.11^{a}	0.007

Notes. Results are presented as means ± *SE* of triplicate observations. Means in the same row with different superscript letters are significantly different ($p < 0.05$).
RBCs, red blood cell count; WBCs, white blood cell count.

TABLE 5 Biochemical blood parameters of *Oreochromis niloticus* 84 days after feeding of yeast extract-supplemented diets

Items	Control	5 g/kg yeast extract	10 g/kg yeast extract	15 g/kg yeast extract	*p* value
ALT (U/L)	90.33 ± 2.18[a]	87.33 ± 2.14[b]	85.30 ± 2.56[c]	84.66 ± 2.16[c]	0.043
AST (U/L)	17.63 ± 0.56[a]	16.83 ± 0.35[b]	15.87 ± 0.58[c]	15.83 ± 0.45[c]	0.0.32
Total protein (g/dl)	3.10 ± 0.41	3.47 ± 0.35	3.67 ± 0.43	3.88 ± 0.55	0.781
Albumin (g/dl)	1.20 ± 0.11[b]	1.28 ± 0.10[b]	1.42 ± 0.09[a]	1.59 ± 0.12[a]	0.034
Globulin (g/dl)	1.90 ± 0.18[b]	2.19 ± 0.13[b]	2.25 ± 0.12[a]	2.29 ± 0.14[a]	0.012
Cholesterol (mg/dl)	95.00 ± 3.13[a]	82.30 ± 2.17[b]	82.33 ± 2.59[b]	79.32 ± 2.22[c]	0.011
Triglycerides (mg/dl)	97.67 ± 2.98[a]	87.33 ± 3.13[b]	82.30 ± 2.18[b]	78.33 ± 2.13[c]	0.012

Notes. Results are presented as means ± *MSE* of triplicate observations. Means in the same row with different superscript letters are significantly different ($p < 0.05$).
ALT: alanine aminotransferase; AST: aspartate aminotransferase.

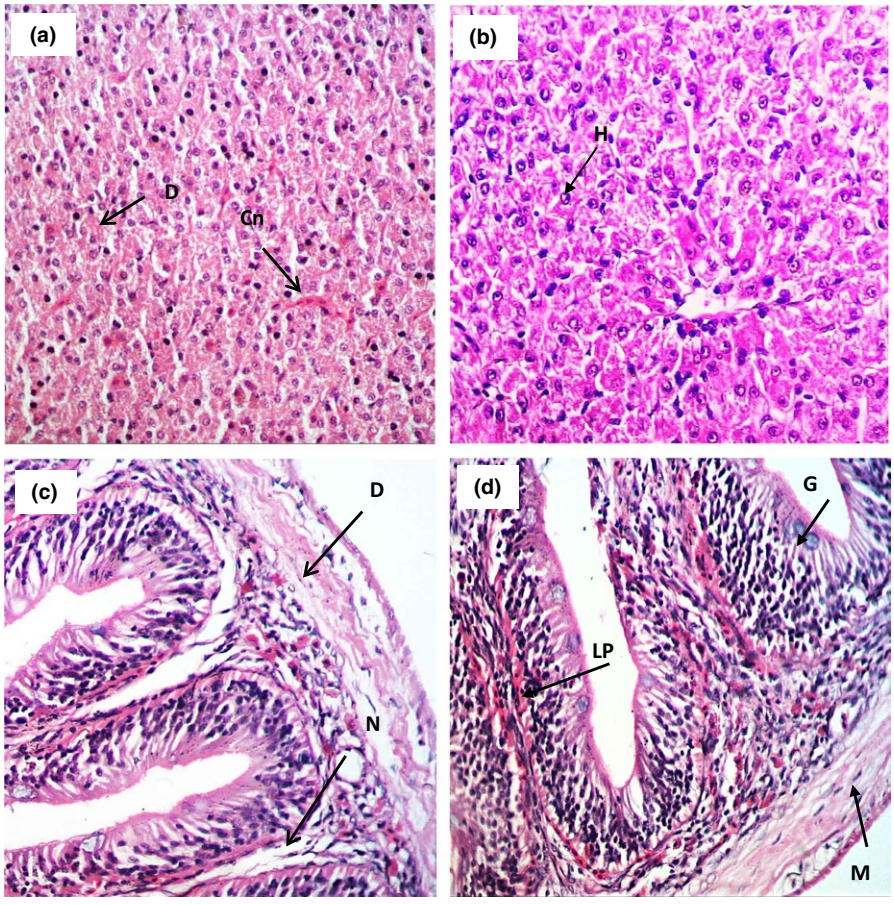

FIGURE 2 Histoarchitecture of liver and intestine tissues of Nile tilapia fed the control diet and diets supplemented with yeast extract: (a) congestion (Cn) in blood sinusoids and degeneration (hypotrophy) (D) of hepatocytes of fish fed the control diet; (b) normal and denser liver morphology of tilapia fed a diet supplemented with yeast extract; (c) degeneration (D) and necrosis (N) in submucosal and mucosal morphology, respectively, of mid-sections of intestine of Nile tilapia fed the control diet; and (d) normal intestine of fish fed a diet supplemented with yeast extract with regular distribution of goblet cells (G) and normal (unbroken) mucosal fold structure and more developed lamina propria (LP) and muscularis (M) layer [Colour figure can be viewed at wileyonlinelibrary.com]

(2003), who supplemented fish diets with nucleotides. Lunger, Craig, and McLean (2006) noted that increasing levels of *S. cerevisiae* extract did not affect nutrient deposition in Nile tilapia. On the other hand, Ebrahimi et al. (2012) demonstrated that a combination of β-glucan and MOS added to diets in the amount of 2.5 g/kg improved the crude protein content in common carp fingerlings.

No significant differences in haemoglobin or haematocrit levels were observed among the fish groups fed diets with yeast extract. Similarly, yeast RNA supplementation had no effect on haematological values of *Labeo rohita* or *Catla catla* (Choudhury et al., 2005; Jha, Pal, Sahu, Kumar, & Mukherjee, 2007). Brewer's yeast extract in doses of 15 g/kg diet significantly enhanced the WBC count in Nile

tilapia blood, which concurs with the study by Jha et al. (2007), who found that there was an increase in leucocyte count when *C. catla* fingerlings were treated with nucleotides. Dietary yeast extract activated other functions of carp leucocytes, including phagocytosis that resulted in an increased phagocytic index value (Biswas et al., 2012). Other research has also shown that exogenous nucleotides can influence both the humoral and cellular components of the innate immune system of common carp (Sakai et al., 2001) and hybrid striped bass (Li et al., 2004). The dietary yeast extract used in the present study caused elevated albumin and globulin levels in serum. Albumin and globulin are essential for a healthy immune system (Tahmasebi-Kohyani et al., 2011). The tilapia diet yeast extract supplement elevated the serum albumin and globulin contents in Nile tilapia. The highest globulin values were noted in *C. catla* fed diets supplemented with yeast RNA, and an increasing trend was noted along with increases in nucleotides (Jha et al., 2007). Also, tilapia fed the diet supplemented with *S. cerevisiae* had a significantly higher level of plasma total protein, albumin and globulin (Goda et al., 2012). On the other hand, El-Boshy, Ahmed, AbdelHamid, and Gadalla (2010) noted a significant increase in cellular and humoral immunity in Nile tilapia that received β-glucan at 0.1% of the diet for 3 weeks. In contrast, Barros et al. (2013) proved that plasma total protein, globulin and albumin in Nile tilapia were not affected by nucleotide levels. Jarmołowicz et al. (2012) also reported that dietary yeast extract had no significant effect on total protein in juvenile pikeperch.

Alanine aminotransferase and AST are important liver enzyme indicators of liver health that control the transfer of amino groups from alpha-amino acids to alpha-keto acids. Thus, high levels of ALT and AST are mostly released into the blood when there is liver cell damage (Racicot, Gaudet, & Leray, 1975). In the present study, lower levels of AST and ALT were noted in fish fed diets supplemented with the highest amount of yeast extract (15 g/kg), which might indicate improved liver function (Metwally, 2009). Similarly, juvenile pikeperch that received yeast extract (40 and 60 g/kg diet) in their diets exhibited significantly lower AST and ALT activity in comparison with the control group (Jarmołowicz et al., 2012). Histological analysis corresponded with the lower liver transaminases noted in the groups that received 15 g/kg yeast extract, which might also indicate improved liver function. Microcopy observations revealed there were normal structures in liver or intestine after yeast extract diet supplementation. Meanwhile, in other analysed variants (even in the unsupplemented control group) slight changes were noted in these organs including degeneration congestion in the blood sinusoids of hepatocytes and degeneration in the hypodermis layer and villi, which often occurs after feeding of high-soybean diets or in intensive fish-farming conditions. This results are in agreement with Hassaan et al. (2014), who reported that liver of Nile tilapia fed control diets which contained high portion of soybean meal was normal architecture, only some samples showed fatty degeneration. A histological analysis of the liver of sea bass fed different levels of yeast extract showed steatosis with fat degeneration, whereas liver morphology was considerably improved with yeast extract supplementation (Panagiotidou et al., 2009). Moreover, nucleotide supplementation significantly increased distal intestine fold height, enterocyte height and microvillus height in juvenile turbot, *Scophthalmus maximus*, compared to the control diet (Peng et al., 2013). In turn, Cheng, Buentello, and Gatlin (2011) noted that dietary nucleotide supplementation significantly improved intestinal structure in red drum. Jarmołowicz et al. (2012) reported that changes in intestinal morphology result in greater cell absorption activity and better digestion of nutrients in the intestine, which usually leads to improved growth performance and feed utilization. The beneficial effects of dietary nucleotides on hepatocytes and the gastrointestinal tract have been more widely investigated in mammals, and nucleotides have been found to improve hepatic function and to promote earlier restoration of nitrogen balance following liver injury (reviewed by Sauer, Bauer, and Mosentih (2009)). Dietary nucleotides have also been noted to repair intestinal mucosa after chronic diarrhoea induced by a lactose-enriched diet in weanling rats fed AMP, GMP, IMP, CMP and UMP (50 mg/100 g each) (Bueno et al., 1994). Nucleotides are partly absorbed in the gut as nucleosides through active transport and Na^+ cotransport and incorporated into body tissues, mainly the liver, spleen, bone marrow and gut (Bueno et al., 1994). Dietary sources of nucleotides might be conditionally essential nutrients. Rapidly growing tissues such as intestinal epithelium or lymphoid cells lack significant capacity for de novo synthesis of nucleotides and require exogenous sources of purine and pyrimidine bases (Uauy, Quan, & Gil, 1994).

Nucleotides can even modify the type and growth of intestinal microflora, especially that of *bifidiobacteria* (Uauy et al., 1994). The present study is the first report on the triglyceride and cholesterol parameters of Nile tilapia fed diets supplemented with yeast extract, and these levels were significantly lower in comparison with the control group. In another 8-week study, rainbow trout fed a diet supplemented with 1–2 g/kg nucleotides exhibited significant decreases in low-density lipoprotein (LDL)-C and triglycerides in comparison with the control fish (Mohebbi, Nematollahi, Gholamhoseini, Tahmasebi-Kohyani, & Keyvanshokooh, 2013). In recent years, dietary nucleotides have been shown to influence lipid metabolism and fatty acids, but the mechanisms by which dietary nucleotides affect circulatory lipid concentrations are not clear. Some researchers believe that dietary nucleotides might increase the synthesis of long-chain polyunsaturated fatty acids possibly by influencing the activity of intestinal and hepatic desaturase enzymes (Gil et al., 1988). For example, feeding a nucleotide-supplemented diet resulted in a significant increase in plasma polyunsaturated fatty acids in mammals (Boza, Jimenez, Faus, & Gil, 1992; Gil, Pita, Martinez, Molina, & Sánchez, 1986; Jiménez, Boza, Suárez, & Gil, 1992).

Generally, brewer's yeast is a particularly important natural bioproduct as it contains immune-stimulating compounds such as β-glucan, nucleotides, mannan oligosaccharides and chitin, and it has been proven to influence the immune response (Abdel-Tawwab, 2012; Abdel-Tawwab, Abdel-Rahman, & Ismael, 2008; Bidhan, 2011; Li et al., 2004). The results of our study indicate that the diets supplemented with yeast extract improved growth and have beneficial effects on haematological and biochemical blood parameters, lipid profiles and the histological structure of liver and gut of Nile tilapia. It can also be used as a source of nucleotides and β-glucan.

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