New Insights into the Effectiveness of Fungi-Degraded Date Pits Supplemented Diet on the Physiological and Biological Indices of *Oreochromis niloticus* Fingerlings

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**ABSTRACT**

This study examined the effects of fungi-degraded date pit (FDDP) as a dietary supplement in fish diets *in vitro* and *in vivo*. In the *in vitro* study, FDDP was prepared and characterized for its nutritional and biological impacts on animal health. A total of nine Nile tilapia (*Oreochromis niloticus*) experimental fish groups were used to assess the sub-chronic toxicity of FDDP crude and ethanolic extract on growth performance and biochemical markers. Four groups of fish (15 ± 0.1 g) received varying concentrations of crude FDDP (5, 10, 20, 30 g/kg diet), while another four received varying concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10 g/kg diet) in addition to the control group. The chemical compositions of FDDP showed high protein, fiber, organic matter, and hemicellulose contents. For the analysis of FDDP, an increase was recorded in mineral contents and xylanase & cellulase enzyme activities, compared to the degraded date pits (DDP). The results of fish biochemical composition and physiological analyses showed that diets containing ethanolic FDDP extract (1%) and crude FDDP (2%) had the highest weight gain (WG), condition factor (K) and protein efficiency ratio (PER) values. Additionally, an increase was detected in the antioxidant capacity by upregulating reduced glutathione (GSH) levels and superoxide dismutase (SOD) activities. In both liver and brain tissues, oxidative stress levels, such as thiobarbituric acid reactive substances (TBARS) and nitric oxide (NO) were declined. In conclusion, our findings indicate that supplementing fish diets with either FDDP ethanolic extract (1%) or crude FDDP (2%) could promote fish growth, increase physiological competence and stress resistance while reducing oxidative stress in the Nile tilapia.

INTRODUCTION

Food loss and waste reduction are important ways to reduce production costs and increase the efficiency of the global food system, as well as contributing to environmental sustainability. As a result from an environmental standpoint, reducing food loss and
waste is expected to have several positive effects, such as improving water management, climate change, marine resources, terrestrial ecosystems, forestry, and biodiversity, among other things.

Several studies have demonstrated that, phytogenic feed additives in fish diets can promote growth, anti-stress, appetite stimulation, hematological and biochemical status in addition to disease resistance (Abo-State et al., 2017; Bharathi et al., 2019). Furthermore, since many fish producers cannot afford adding expensive feed additives to fish diets (Hass et al., 2016); nowadays, the use of organic feed additives to improve feed use efficiency in aquaculture has become a critical demand (Tibbetts et al., 2017).

*Phoenix dactylifera* L. is a major fruit crop in the Middle East and the Mediterranean region. According to the Food and Agriculture Organization (FAO, 2011), Egypt produces 1330,000 ton per year, ranking the second to China. Egypt's dates accounted for roughly 17% of global production. Date seeds (date pits) make up 10- 20% of the date fruit. Date pits are rich in protein, fat, dietary fiber and phenolic acids (Attia et al., 2021). In addition, they can be used to boost the nutritional value of food products (Guizani et al., 2014; Tafti et al., 2017).

Date pits are currently used as a feed additive for sheep (Aldosari et al., 1995), broilers (Alyileili et al., 2020) and fish (Dawood et al., 2020). Date pits are rich in bioactive non-nutrient compounds called phytochemicals, a secondary plant metabolite in addition to nutrient-rich compounds (Bouhlali et al., 2020).

Date pits contain high amounts of crude protein, fiber, neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Rahman et al., 2007), which limit their use as a feed ingredient. Fish (animals) can better use date pits if specific enzymes degrade these fibers into simpler carbon molecules. Xylanase enzymes are used in food formulation to hydrolyze or release nutrients blocked by non-degradable cellulose and hemicellulose (Leisola et al., 2002). This increases food consumption while decreasing the amount of animal feces produced. The degradation of dietary fiber by microorganisms producing xylanase enzymes is cheaper than using enzymes in animal feed. *Trichoderma reesei* (also known as *Hypocrea jecorina*) is the most studied cellulolytic fungi (Foreman et al., 2003). The U.S. Food and Drug Administration has approved using it for animal (fish) feed (Food & Drug Administration (FDA), 1993). It efficiently produces xylanases and cellulase-degrading enzymes in large quantities (Fang et al., 2021). Additionally, the degradation of date pits improves the chemical constituents and produces bioactive substances with added value for animal nutrition (Alyileili et al., 2020).

The present study aimed to produce a safe, low-cost byproduct with high biological impact as a supplement in fish diets, focusing on the investigation of its biochemical and physiological effects on *Oreochromis niloticus*. 
2. Materials and methods of the study

2.1. Degraded date pits (DDP)

To avoid sticking dirt, the date pits (Phoenix dactylifera L.) were purchased from El-Wadi El Gedid Factory for Packaging Dates (El-Wadi El Gedid, Egypt). They were washed with regular tap water and dried for 8 hours at 80°C. Date pits were finely ground using a feed grinder, sieved through 0.6mm sieves and stored at –20°C until used. *Trichoderma reesei* fungi (AUMC 5829) were purchased from Assiut University Mycological Center AUMC, Assuit, Egypt. Half of the grinded date pits was degraded. The chemical and nutritional compositions of both DDP and FDDP were determined prior to the preparation of the test diets (Table 1).

2.2. *Trichoderma reesei*—fungi culture

Ampoules of dried (vacuum) *Trichoderma reesei* fungi culture (AUMC 5829) were rehydrated with 25ml of potato dextrose agar media (PDA; potato infusion 200g, agar 20g, and dextrose 20g in 1L distilled water, with pH value of 5.6±0.2, then a sub-culture of *Trichoderma reesei* fungi was transferred to grow on PDA plates at 30°C for seven days in the dark.

2.3. Production of fungi-degraded date pits (FDDP)

For solid state fermentation (SSF), autoclavable bags were used to produce fungi-degraded date pits. Initial cleaning and sterilization of glassware as well as substrating DDP at 12°C for 20 min were performed, followed by media (one liter of culture media contained 1% malt extract, 1.89 mM (NH₄)₂SO₄, 10.29 mM KH₂PO₄, 18.02 mM CaCl₂, 1.21mM MgSO₄.7H₂O, 35.29 mM NaNO₃, 6.70 mM KCl, 2.01 μM CuSO₄.5H₂O, 1.79 μM FeSO₄.7H₂O, 7.17 μM MnSO₄.4H₂O, 4.86 μM ZnSO₄.7H₂O, 84.05 μM CoCl₂.6H₂O and 0.1% tween 80 (v/v), pH 5.5 and 2.5% lactose). Next, eight 6mm agar plugs from *Trichoderma reesei* active margins were inoculated with half a kilogram of degraded date pits (DDP). The autoclavable bags were incubated at 28°C for two weeks. Since the process was mostly aerobic, constant aeration was required. The fungi degraded date pits (FDDP) were autoclaved twice and stored at –20°C. A series of tests confirmed the formation of FDDP and the action of *Trichoderma reesei* fungi over DDP.

2.4. Determination of chemical compositions of samples

Samples (DDP and FDDP) were analyzed for contents of crude protein (CP), ash, crude fat, NDF, acid detergent fiber (ADF) and acid detergent lignin (ADL). Dry matter (DM) was determined after drying at 105°C and ash after combustion at 550°C. Crude fat was extracted for 6h with petroleum ether; whereas, the Kjeldahl method was used to
Effect of Fungi- Degraded Date Pits Supplemented Diet on Oreochromis niloticus

determine nitrogen (N) (AOAC, 1990). CP was calculated as N × 6.25. NDF, ADF, and ADL were determined according to the methods of Van Soest et al. (1991), using an ANKOM 220 fiber analyzer (ANKOM Technology Corporation, NY, USA). Hemicellulose was calculated as NDF – ADF and cellulose was evaluated as ADF – ADL (Rinne et al., 1997).

2.5. Sample preparation for mineral analysis

The samples (DDP and FDDP) were analyzed in triplicate for the macro and microelements as nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu), using a NOUVA400 atomic absorption spectrophotometer (Analytik Jena, Germany) (American Public Health Association [APHA] et al., 1999).

2.6. Morphological characterization

In this study, scanning electron microscope (SEM) was utilized to examine the surface morphology of degraded date pits before and after fermentation with Trichoderma reesei fungi. Gold sputtering was used to ensure surface conductivity. The SEM conditions for secondary electrons used were: 15 kV beam intensity acquiring 1,500 X magnification (Kalantar-zadeh & Fry, 2008).

2.7. Crude enzymes extraction

An enzyme extraction from FDDP was compared to DDP after 5 days, 1 week and 2 weeks of inoculation. An hour was needed to extract enzymes from 10g of powder in a 50ml buffer solution (Citrate phosphate, 0.1 M buffer; pH 5.0). The extract was filtered through damp cheesecloth and centrifuged at 805xg for 15min to obtain extracellular enzymes (Shamala & Sreekantiah, 1987). Cellulase was converted to cellulose and then to glucose (King & Vessal, 1969). The released glucose forms a red complex with dinitrosalicylic acid (DNS) at 575nm (Miller, 1959).

2.8. In vitro analyses of ethanolic and aqueous extracts of DDP and FDDP

2.8.1. Preparation of aqueous and ethanolic extracts. Each sample (1g) was extracted twice by stirring with 30ml of 70% ethanol. For the aqueous extract, a decoction was prepared by boiling 100g of DDP and FDDP powder in one liter of distilled water for 15min, and then the mixture was filtered, lyophilized and stored at –20˚C for further analysis.

2.8.2. Phytochemical investigations of DDP and FDDP extracts. Total phenolics content of DDP and FDDP aqueous and ethanolic extracts were spectrophotometrically assessed following the Folin–Ciocalteu method (Slinkard & Singleton, 1977). Total flavonoid was analyzed by aluminum chloride colorimetric method (Chang et al., 2002).
2.8.3. *In vitro* biological screening. The 2,2-diphenyl-1-picryl hydrazyl (DPPH) scavenging activity was assessed according to the study of Blois (1958). Further, the nitric oxide (NO) radical scavenging activity was evaluated according to Garrat (1964).

2.8.4. Determination of cellular cytotoxicity of DDP/FDDP extracts. To begin with, a sterile heparin tube was used to isolate and culture human white blood cells (WBCs). “N” represents number of viable or nonviable cells and “D” represents sample dilution (1:1 with the trypan blue). For using the cells for testing, at least 90% must be viable. Then, different extracts were tested on WBCs in 96-well plates with 100μL of cell suspension (1 × 10⁵ cells/ ml). The plate was incubated at 37°C for 24h, with 5% humidified CO₂. After collecting a semi-conflict cell layer, an amount of 100μL of various DDP and FDDP extracts was applied (12.5, 25.50, 100, 200 g/ml), and media (control) were incubated for three days at 37°C. Afterward, each cell layer received 100μL of working solution (40μg/ ml) and incubated for three hours at 37°C in 5% CO₂. Hundred microliters of fixation solution (0.5% formalin with 1% calcium chloride) was applied for one minute and 0.5% formalin, with 1% calcium chloride fixation for one min, followed by 5min in a destaining solution (50% ethanol with 1% glacial acetic acid). Assuring a percentage>85 of cell viability was determined by measuring stain intensity at 540nm, using ELISA microplate reader. The percent of cell viability= AT/AC ×100; where, AT: the mean absorption of the treatment cells at various concentrations of each extract and [AC = the mean of absorbance of control untreated cells only with culture medium] (Repetto et al., 2008).

2.8.5. Quantitative determination of DDP and FDDP ethanolic fractions contents by high performance liquid chromatography (HPLC). The analysis of the ethanolic fractions of DDP and FDDP extracts were carried out on an Agilent Technologies 1100 HPLC system fitted with an auto sampler and diode array detector. The analytical column was Eclipse XDB-C18, with a C18 guide (Phenomenex, Torrance, CA) column (150 X 4.6μm; 5μm). The mobile phase was acetonitrile (solvent A) acetic acid (v/v) of 2% (solvent B). The flow rate was held at 0.8ml/ min for 60min, with the gradient set to: 100% B to 85% B in 30min, 85% B to 50% B in 20min, 50% B to 0% B in 5min, and 0% B to 100% B in 5min. The amount of injections was 50μL, and the peaks were simultaneously tracked for benzoic acid and cinnamic acid derivatives at 280 and 320nm, respectively, in addition to flavonoids at 360nm. DDP and FDDP fractions identification was made by comparing the relative retention times of sample peaks with those of the reference standards (Gerber et al., 2004).

2.9. Experimental diets
The present study evaluated nine isonitrogenous (29%, CP), isoenergetic (18 MJ/kg) experimental diets for the Nile tilapia (*Oreochromis niloticus*) fingerlings (Table 1). The experiment was designed as follows: a control basal diet (D0), four isonitrogenous-isoenergetic diets containing graded concentrations of FDDP ethanolic extract as a feed
additive (2.5, 5, 7.5, 10g of FDDP/ kg diet), which were designated as D1, D2, D3, and D4, and four isonitrogenous-isoeenergetic diets containing different concentrations of crude FDDP as a feed supplement (5, 10, 20, 30g of FDDP/ kg diet). All dry ingredients were grinded, screened, and mixed with a mixer. The oil was slowly added to the vitamins and minerals mixture with a continuous mixing and then added to the ingredients. The warm water was slowly added to form dough, which was then minced into pellets. Pellets were dried in a forced air-drying oven at 60°C for 24h. They were kept in dry, sealed bags at 20°C until needed. The test diets’ biochemical composition was determined in triplicate using standard methods (AOAC, 1995).

Table 1. Ingredients and proximate composition (% DM) of control diet and the two forms of FDDP supplemented diets fed to the Nile tilapia (Oreochromis niloticus)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>D0</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal1</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Wheat bran</td>
<td>170</td>
<td>170</td>
<td>170</td>
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<td>170</td>
<td>170</td>
<td>170</td>
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<tr>
<td>Wheat flour</td>
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<td>145</td>
<td>142.5</td>
<td>140</td>
<td>145</td>
<td>140</td>
<td>140</td>
<td>130</td>
<td>120</td>
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<td>Sunflower oil</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Vitamins &amp; minerals Premix3</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
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<tr>
<td>FDDP extract</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>FDDP crude</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Biochemical composition (% DM)

<table>
<thead>
<tr>
<th></th>
<th>D0</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>0.48</td>
<td>90.89</td>
<td>90.89</td>
<td>90.59</td>
<td>90.99</td>
<td>89.99</td>
<td>89.89</td>
<td>89.89</td>
<td>89.89</td>
</tr>
<tr>
<td>Fiber</td>
<td>3.66</td>
<td>3.85</td>
<td>3.49</td>
<td>3.49</td>
<td>3.49</td>
<td>3.49</td>
<td>3.49</td>
<td>3.49</td>
<td>3.49</td>
</tr>
<tr>
<td>NFE4</td>
<td>2.26</td>
<td>51.44</td>
<td>51.72</td>
<td>51.44</td>
<td>51.11</td>
<td>51.71</td>
<td>51.69</td>
<td>51.69</td>
<td>51.69</td>
</tr>
</tbody>
</table>

1 lab made (58% protein), 2(42% protein), 3Vitamins and minerals premix (mg kg^-1): p-amino benzoic acid (9.48); D-biotin (0.38); inositol (379.20); niacin (37.92); Ca panthothenate (56.88); pyridoxine HCl (11.38); riboflavin (7.58); thiamine HCl (3.79); L-ascorbyl-2-phosphate Mg (APM) (296.00); folic acid (0.76); cyanocobalamine (0.08); menadione (3.80); vitamin A palmitate (17.85); α-tocopherol (18.96); calciferol (1.14). K3PO4 (2.01); Ca3 (PO4)2 (2.736); Mg SO4·7H2O (3.058); NaH2PO4·2H2O (0.795). 4Nitrogen-free extracts (NFE) = 100 − [% ash + % lipid + % protein + % fiber]. 5Gross energy was quantified based on 23.6, 39.5 and 17.2 kJ/g for protein, lipid and carbohydrates, respectively (NRC, 1993).

2.10. Fish and experimental design

Five hundred Nile tilapia (Oreochromis niloticus) fish were purchased from a private farm and transported to the lab. For adaptation, fish were placed in 3m^3 fiberglass tank and fed on commercial diet for a week (30% crude protein and 8% lipid, Aller Aqua, Egypt). Nine dietary fish groups were identified in triplicate and randomly distributed...
into 27 (200L) aquariums, with a stocking density of 15 fish per aquarium and an initial body weight of 15g/ fish.

The culture tanks were provided with air stones and about 30% of their water was replaced daily with similar-temperature fresh water. Throughout the trial, the culture unit was lit at 12:12 12:12-h light: dark cycle. Water quality parameters, including temperature, pH and dissolved oxygen were weekly measured, using a Hanna HI-9828 portable water quality meter, while salinity was measured using a Hanna GG-201/211 portable refractometer. Fish were hand- fed three times per day with 4% of fish body weight at 08:00am, 13:00pm and 17:00pm; whereas, the uneaten food was siphoned and quantified after each meal. Upon completing the feeding trial, each fish tank was netted, counted, and weighed collectively. Ten fish from each tank were rinsed with distilled water, dried with a paper towel, and their weights (g) and lengths (cm) were measured. The remaining fish were frozen at –20˚C for further analyses.

2.11. Evaluation of growth performance, feed utilization and biometric indices
Fish growth performance and feed utilization were analyzed in terms of survival (S%), weight gain (WG, g), percentage weight gain (%WG), specific growth rate (SGR, %), feed conversion ratio (FCR) and protein efficiency ratio (PER). The following formulae were used: Survival (S%) = (final fish count/ initial fish count) × 100, WG (g) = final fish weight (FW) (g) – initial fish weight (IW) (g); WG (%) = 100 × [(FW – IW)/IW]; SGR = 100 (Ln FW) – (Ln IW)/experimental days); FCR = feed fed (g)/weight gain (g), and PER= weight gain (g)/protein fed (g). The measured biometric indices were the hepatosomatic (HSI) and viscerosomatic (VSI) indices and the condition factor (K). Hepatosomatic index (HSI) = 100× [liver weight (g)/ body weight (g)] and viscerosomatic index = 100 × [viscera weight (g)/body weight (g)]. Condition factor (CF) = 100 × [body weight (g)/ length³ (cm)].

2.12. Fish biochemical analyses
The proximate composition of the whole fish body was estimated in triplicate samples. The contents of moisture, lipid, protein, and ash were all analyzed using the methodology of AOAC (1995).

2.13. Blood samples
Immediately, at the end of the experiment, five fish per tank were randomly sampled for blood collection (15 fish per treatment), using non-heparinized syringes from the caudal vasculature, pooled for each tank and centrifuged at 3500xg for 10min. Serum samples were kept at –20˚C until analysis.

2.14. Routine blood analyses
Serum and blood analyses of uric acid, creatinine, glucose, alanine amino transferase (ALT), aspartate amino transferase (AST) and hemoglobin content (HB) were measured using commercial kits (Biotec, Egypt).
2.15. Preparation of tissue samples for oxidant and antioxidant biomarkers

The fish samples were weighed, decapitated, and dissected on ice. Liver and brain tissues were homogenized with nine volumes of potassium phosphate buffer, 0.1 M, pH 7.4, and then centrifuged at 35000 x g for 15 min, and the supernatant was kept at 4°C to be subsequently used to measure the levels of thiobarbituric acid reactive substances (TBARS) (Tappel & Zalkin, 1959), nitric oxide (NO) (Montgomery & Dymock, 1961), superoxide dismutase (SOD) and the reduced glutathione (GSH) (Marklund & Marklund, 1974). The acetylcholinesterase (AChE) activity in brain tissue was measured following the method of Jollow et al. (1974).

2.16. Statistical analysis

All data were subjected to one-way ANOVA, followed by the Tukey’s test to assess significant differences among groups at \( P < 0.05 \). All the statistical analyses were done using the SPSS program version 20 (SPSS, Richmond, VA, USA) as described in the study of Dytham (2011).

Results

3. Current study results

3.1. Chemical compositions of samples

Table (2) compared the proximate compositions of FDDP to DDP throughout the time intervals of FDDP preparation, where day zero (Day 0) represents the start of inoculation or DDP; week one represents the 7th day after inoculation, and week two stands for day 14 after inoculation (FDDP formation). Dietary crude protein, fiber, organic matter and nitrogen free extract were higher in FDDP than in DDP.

<table>
<thead>
<tr>
<th>Time interval</th>
<th>Moisture</th>
<th>Organic matter</th>
<th>Crude protein</th>
<th>Crude fiber</th>
<th>Ether extract</th>
<th>Nitrogen free extract</th>
<th>Ash</th>
<th>NDF</th>
<th>ADF</th>
<th>ADL</th>
<th>Hemicellulose</th>
<th>Cellulose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>3.12±0.51b</td>
<td>98.46±0.91b</td>
<td>8.84±0.51b</td>
<td>7.30±0.67b</td>
<td>1.32±0.71b</td>
<td>81.22±0.91b</td>
<td>1.32±0.89b</td>
<td>26.60±0.74d</td>
<td>32.01±0.43b</td>
<td>5.63±0.88b</td>
<td>81.1±0.11b</td>
<td>22.9±0.51b</td>
</tr>
<tr>
<td>Week One</td>
<td>3.41±0.32a</td>
<td>98.51±0.85b</td>
<td>13.8±0.21a</td>
<td>8.03±0.61a</td>
<td>1.44±0.58a</td>
<td>74.89±0.66b</td>
<td>1.94±0.14a</td>
<td>27.80±0.32b</td>
<td>31.10±0.11b</td>
<td>6.61±0.59b</td>
<td>11.50±0.38b</td>
<td>21.9±0.19b</td>
</tr>
<tr>
<td>Week Two</td>
<td>3.42±0.41a</td>
<td>98.68±0.78b</td>
<td>14.63±0.77a</td>
<td>7.83±0.38a</td>
<td>1.36±0.68a</td>
<td>75.06±0.45b</td>
<td>2.01±0.76a</td>
<td>36.70±0.93b</td>
<td>28.5±0.57b</td>
<td>6.64±0.65b</td>
<td>13.96±0.94a</td>
<td>21.5±0.33a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean ± SE. Means with different letters represent significant difference \( P < 0.05 \) within the same column, the mean with letter (a) was the highest value.
3.2. Mineral analysis of samples

Table (3) shows FDDP containing a two-fold positive increment of nitrogen, phosphorous, and potassium, compared to DDP. Moreover, FDDP had higher concentrations of calcium, magnesium, iron, and zinc, but lower concentrations of manganese and copper, compared to DDP (Day 0) at $P<0.05$.

### Table 3. Mineral analysis of FDDP and DDP

<table>
<thead>
<tr>
<th>Time</th>
<th>N%</th>
<th>P%</th>
<th>K%</th>
<th>Ca (μg/g)</th>
<th>Mg (μg/g)</th>
<th>Fe (μg/g)</th>
<th>Zn (μg/g)</th>
<th>Mn (μg/g)</th>
<th>Cu (μg/g)</th>
<th>P (μg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1.32±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.28±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>34.11±1.47&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.70±1.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.04±0.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.90±0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.06±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.13±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week one</td>
<td>2.54±0.33&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.46±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.33±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>53.04±1.51&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.97±1.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.49±0.37&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.38±0.72&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.10±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.05±0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.36±0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week two</td>
<td>2.57±0.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.41±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.98±0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.17±1.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.44±0.73&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.48±0.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.11±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08±0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.27±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P<0.05$) within the same column; the mean with letter (a) was the highest value.

3.3. Morphological characterization

Fig. (1a, b) shows transversal sections of date pit particles before and after fungi degradation. Fig. (1a) presents transversal sections of degraded date pit (DDP) particles before fungi degradation, revealing thick tubes with a solid surface. While Fig. (1b) displays transversal sections of date pits during fungi degradation (FDDP), where different stages of *Trichoderma reesei* fungi colonies, date pit microscopic tubes were separated from each other, and particle sizes started getting smaller and looser as the degradation process progressed.

3.4. Fungus enzymatic specific activities during FDDP preparation time course

Table (4) reveals the carboxy methyl cellulase and xylanase enzymes' specific activities throughout the experimental time course. Both enzymes’ specific activities were significantly increased ($P<0.05$) by time till reaching their highest activities on the 14<sup>th</sup> day of inoculation, indicating that the degradation process was in progress, and FDDP was fully formed.

### Table 4. Fungus enzymatic specific activities

<table>
<thead>
<tr>
<th>Enzyme/Time interval</th>
<th>Carboxy methyl cellulase specific activity (IU/mg protein)</th>
<th>Xylanase enzyme specific activity (IU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day zero (DDP)</td>
<td>1.17±0.01&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.45±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fifth Day</td>
<td>1.58±0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.20±0.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week one</td>
<td>1.74±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.36±0.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Week two</td>
<td>2.48±0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.46±0.25&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values were expressed as Mean ± SE. Means with different letters represent significant difference ($P<0.05$) within the same column; the mean with letter (a) was the highest value.
3.5. Phytochemical compositions of DDP and FDDP extracts

As shown in Fig. (2), the highest values of total phenolics and flavonoids content were found in FDDP ethanolic extract (26.52 and 2.11 mg/100g DW, respectively), followed by FDDP aqueous extract (25.67 and 1.97 mg/100g DW, respectively).

![Graph of flavonoids and phenolics concentrations](image-url)
3.6. The antioxidant activities of DDP and FDDP extracts

Table (4) shows the free radical scavenging activity using DPPH, and the nitric oxide scavenging activity of DPP and FDDP in aqueous and ethanolic extracts. Both DDP and FDDP extracts were radical scavengers. The radical scavenging activity of DDP "aqueous extract" increased from 15.89–51.28% as concentrations increased from 1–100 mg/mL, respectively; while, FDDP "aqueous extract" increased from 28.97–59.69%. Scavenging activity increased significantly (P< 0.05) in the range of 16.29–51.53%, respectively, in the case of DDP "ethanolic extract" and from 27.45–55.48% in the case of FDDP "ethanolic extract". However, their activities didn’t exceed the antioxidant activity of the ascorbic acid (as a positive control), which showed the highest DPPH scavenging activity (87%) at a minimal concentration of 1mg/ml. A reduction in nitric oxide radical formation indicates anti-inflammatory activity. Based on Table (5), both the FDDP extracts had higher anti-inflammatory activities compared to the DDP ones at all concentrations.

Table 5. The antioxidant and anti-inflammatory effect of DPP and FDDP extracts

<table>
<thead>
<tr>
<th>Concentrations (mg/mL)</th>
<th>%Radical scavenging activity</th>
<th>%NO scavenging activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aqueous extracts</td>
<td>Ethanollic extracts</td>
</tr>
<tr>
<td></td>
<td>DDP</td>
<td>FDDP</td>
</tr>
<tr>
<td>1</td>
<td>15.89±2.21&lt;sup&gt;c&lt;/sup&gt;</td>
<td>28.97±3.41&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>45.15±1.61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>31.79±3.50&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>49.74±2.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.69±2.12&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>51.28±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.69±3.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IC&lt;sub&gt;50&lt;/sub&gt; (mg/ml)</td>
<td>3.35±0.98</td>
<td>2.98 ±1.1</td>
</tr>
</tbody>
</table>

All values were expressed as Mean ± SE. Means with different letters represent significant difference (P< 0.05) within the same column; the mean with letter (a) was the highest value.

3.7. Cellular cytotoxicity assessment of DDP/FDDP extracts

Aqueous DDP extract (Fig. 3a), aqueous FDDP extract (Fig. 3b), ethanolic DDP extract (Fig. 3c) and ethanolic FDDP extract (Fig. 3d) had no cytotoxic effect on human WBCs up to 200μg/ ml. FDDP ethanolic extract was the safest one because it exerted the highest IC<sub>50</sub> value (614.58μg/ ml), followed by DDP ethanolic extract (368μg/ ml), and finally DDP aqueous extract (255.77μg/ ml) at P< 0.05.
Effect of Fungi-Degraded Date Pits Supplemented Diet on *Oreochromis niloticus*

(a) $IC_{50}=255.77\pm14 \mu g/ml$

(b) $IC_{50}=327.23\pm20 \mu g/ml$

(c) $IC_{50}=368.10\pm22 \mu g/ml$
Fig. 3. Cell viability of WBCs treated with DDP and FDDP aqueous and ethanolic extracts after 72 hours

(a) WBCs treated with DDP aqueous extract, (b) WBCs treated with DDP ethanolic extract, (c) WBCs treated with FDDP aqueous extract, (d) WBCs treated with FDDP ethanolic extract.

3.8. HPLC polyphenols profile of DDP and FDDP ethanolic extracts

Results in Table (6) show the HPLC analysis of bioactive compounds extracted from DDP and FDDP 70% ethanolic extracts, where retention times and UV spectra were compared to standards (Supplementary Fig. 1). Chrysin, naringenin, luteolin, apigenin-7-O-Glucoside, sinapic acid, and protocatechuic acid were found at the highest concentrations in the FDDP ethanolic extract. Moderate concentrations were detected in gallic, caffeic, rosmarinic, cinnamic, kaempferol, and the lowest concentrations of catechin, vanillic, and p-coumaric were recorded, compared to DDP ethanolic extract.

Table 6. HPLC identity of bioactive compounds present in DPP and FDDP ethanolic extracts

<table>
<thead>
<tr>
<th>Compound</th>
<th>Sample/ concentration (μg/g)</th>
<th>DDP ethanolic extract</th>
<th>FDDP ethanolic extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallic</td>
<td>11.734</td>
<td>32.196</td>
<td></td>
</tr>
<tr>
<td>Protocatechuic</td>
<td>75.125</td>
<td>210.152</td>
<td></td>
</tr>
<tr>
<td>p-hydroxybenzoic</td>
<td>2796.922</td>
<td>1363.961</td>
<td></td>
</tr>
<tr>
<td>Catechin</td>
<td>53.414</td>
<td>11.482</td>
<td></td>
</tr>
<tr>
<td>Caffeic</td>
<td>10.021</td>
<td>17.938</td>
<td></td>
</tr>
<tr>
<td>Syringic</td>
<td>17.974</td>
<td>154.427</td>
<td></td>
</tr>
<tr>
<td>Vanillic</td>
<td>30.451</td>
<td>19.750</td>
<td></td>
</tr>
<tr>
<td>Ferulic</td>
<td>2.277</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Sinapic</td>
<td>292.374</td>
<td>467.276</td>
<td></td>
</tr>
<tr>
<td>p-coumaric</td>
<td>18.679</td>
<td>5.091</td>
<td></td>
</tr>
<tr>
<td>Apigenin-7-O-Glucoside</td>
<td>348.032</td>
<td>533.262</td>
<td></td>
</tr>
<tr>
<td>Rosmarinic</td>
<td>63.432</td>
<td>72.842</td>
<td></td>
</tr>
<tr>
<td>Cinnamic</td>
<td>8.443</td>
<td>10.762</td>
<td></td>
</tr>
<tr>
<td>Luteolin</td>
<td>62.781</td>
<td>95.285</td>
<td></td>
</tr>
<tr>
<td>Naringenin</td>
<td>ND</td>
<td>255.963</td>
<td></td>
</tr>
<tr>
<td>Apigenin</td>
<td>41.960</td>
<td>31.851</td>
<td></td>
</tr>
<tr>
<td>Kaempferol</td>
<td>5.148</td>
<td>7.310</td>
<td></td>
</tr>
<tr>
<td>Chrysin</td>
<td>1.927</td>
<td>37.384</td>
<td></td>
</tr>
</tbody>
</table>
3.9. Growth performance, feed utilization efficiency and biometric indices

Table (7) shows the results for the growth and feed utilization indices. Diets supplemented with D4 (1%) and D7 (2%) had the highest WG values (10.54% and 10.49%, respectively), except for D5 (0.5% crude FDDP; 0.94%) and D6 (1% crude FDDP; 0.950.15) diets, which showed a significant decrease in SGR levels when compared to the control group (D0) at P<0.05. A non-significant difference in FCR level was detected at P 0.05 between different groups and the control one, except for D4 (1% ethanolic extract; 2.17%), D7 (2% crude FDDP; 2.12%) and D8 (2% crude FDDP; 2.25%) diets, which showed the lowest FCR levels (P<0.05). All diets supplemented with FDDP ethanolic extract (D1-D4) and crude FDDP (D5-D8) showed non-significant differences in PER values, compared to the control group at P<0.05, except for D5, which recorded the lowest significant (P<0.05) PER value, compared to all the experimental groups.

At all inclusion levels, the use of FDDP, either in crude or ethanolic extract form, resulted in a significant increase (P<0.05) in HSI values relative to the control group, with the exception of the D4 group, which showed insignificant variation with the control (D0) group (P>0.05). Moreover, values of VSI showed a significant increase in all dietary fish groups, except for D4 and D7, which were insignificantly different from D0 (P>0.05). Similarly, the condition factor (K) values significantly increased (P<0.05) directly relative to the dietary supplementation levels of either FDDP crude or ethanolic extract, showing the highest condition factor value in the D4 and D7 experimental groups, and only the D1 group showed a significant difference, compared to the control (D0) group.

Table 7. Fish performance indices of Oreochromis niloticus at the end of the experimental feeding trial

<table>
<thead>
<tr>
<th>Growth and feed utilization indices</th>
<th>Experimental diets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D0</td>
</tr>
<tr>
<td>IW (D0)</td>
<td>14.77±0.55</td>
</tr>
<tr>
<td>WG</td>
<td>9.99±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SGR</td>
<td>1.15±0.22&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>FI</td>
<td>24.06±0.75</td>
</tr>
<tr>
<td>FCR</td>
<td>2.39±0.21&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>PER</td>
<td>1.49±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>HSI</td>
<td>1.78±0.02&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>VSI</td>
<td>7.19±0.59&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>K</td>
<td>1.60±0.01&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values were expressed as Mean ± SE. Means with different letters represent significant difference (P<0.05) within the same column; the mean with letter (a) was the highest value.

D0, Control diet; D1-D4: Diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/kg diet), respectively; D5- D8: Diets containing different concentrations of crude FDDP (5, 10, 20, 30g/kg diet), respectively. WG: Weight gain (g); FCR, Food conversion ratio; FI, Feed intake (g); HSI: Hepatosomatic index; IW: Initial body weight (g); K, Condition factor; PER, Protein efficiency ratio; SGR, Specific growth rate (%/day); VSI, Viscerosomatic index.
3.10. Fish biochemical composition

The carcass biochemical composition measured at the end of the experiment is presented in Table (8). The results showed that fish fed either D4 or D7 diets had significantly higher protein content than all other tested fish groups \((P<0.05)\), with the exception of the control group \((16.89\%)\), where their protein contents were insignificantly different \((P>0.05)\). On the other hand, results of fish biochemical composition indicate insignificant variation among all dietary groups \((P>0.05)\) in the lipid content. An increase in fish moisture content was recorded upon feeding fish with FDDP either crude or ethanolic extract in comparison with the control group, except in the D4 and D7 groups where moisture contents \((74.21\%\) and \(74.17\%\), respectively) were insignificantly different \((P>0.05)\) relative to the D0 group \((74.65\%)\). Except for the D8 diet group, which showed an insignificant difference with the control \((D0)\) group, FDDP in both forms and inclusion levels increased the ash content relative to the control fish group \((P<0.05)\).

![Table 8](image_url)

Table 8. Biochemical composition of *Oreochromis niloticus* at the end of the experimental feeding trial

<table>
<thead>
<tr>
<th>Biochemical composition (%)</th>
<th>DO</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>16.89±0.13&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>16.11±0.66&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.13±0.06&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.18±0.22&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.08±0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.10±0.07&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>16.23±0.42&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.25±0.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.25±0.53&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Crude lipid</td>
<td>4.6±0.148&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.67±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.70±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.80±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.84±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.63±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.69±0.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.72±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.82±0.23&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture</td>
<td>74.65±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.26±1.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.18±0.18&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.09±0.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.21±0.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.22±0.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.01±0.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>74.17±0.73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>75.27±0.90&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ash</td>
<td>3.25±0.08&lt;sup&gt;d&lt;/sup&gt;</td>
<td>3.48±0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.49±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.43±0.05&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.34±0.05&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.56±0.07&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.57±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.38±0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2±0.081&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values were expressed as Mean ± SE. Means with different letters represent significant difference \((P<0.05)\) within the same column, the mean with letter \((a)\) was the highest value.

D0, Control diet; D1-D4, Diets containing different concentrations of FDDP ethanolic extract \((2.5, 5, 7.5, 10\, \text{g/kg diet}),\) respectively; D5-D8: Diets containing different concentrations of crude FDDP \((5, 10, 20, 30\, \text{g/kg diet},\) respectively).

3.11. Effect of FDDP supplementation on biochemical analyses of *O. niloticus*

Table (9) indicates that all experimental groups \((D0-D8)\) had hyperglycemia except for D5, which had hypoglycemia and D4, which was normoglycemic \((P<0.05)\). All experimental groups showed significant increase in uric acid and creatinine levels at \(P<0.05\), compared to the control group but still in the non-chronic range of values, while the D4 and D7 groups showed a significant decrease at \(P<0.05\), compared to all groups. Identically, the ALT activity \((\text{U/L})\) of the groups of D1, D2, D3, D5, D6 and D8 was significantly higher than the control group \((D0)\). The ALT activity \((\text{U/L})\) was lowest in groups D4 and D7 \((p<0.05)\). Moreover, different experimental diets supplemented with variant concentrations of FDDP crude and ethanolic extract showed either the same or lower AST activities when compared to the D0 control group, especially the D4 and D7 groups, which had the lowest activities at \(p<0.05\).
Table 9. Effect of FDDP supplementation on biochemical analyses of *O. niloticus*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>D0</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>D4</th>
<th>D5</th>
<th>D6</th>
<th>D7</th>
<th>D8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>61.90±1.21^e</td>
<td>67.45±2.35^d</td>
<td>156.85±1.56^a</td>
<td>98.71±2.73^b</td>
<td>62.78±2.26^c</td>
<td>31.47±1.81^f</td>
<td>93.95±2.52^b</td>
<td>85.95±1.89^c</td>
<td>73.42±1.46^d</td>
</tr>
<tr>
<td>HB (g/dl)</td>
<td>6.09±0.13^d</td>
<td>6.11±0.10^f</td>
<td>5.84±0.18^d</td>
<td>6.77±0.01^c</td>
<td>9.80±0.51^d</td>
<td>7.11±0.27^c</td>
<td>6.30±0.02^d</td>
<td>10.18±0.07^a</td>
<td>6.54±0.13^b</td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>9.65±0.91^d</td>
<td>17.41±1.01^b</td>
<td>12.10±0.31^d</td>
<td>15.94±1.45^d</td>
<td>9.65±0.61^d</td>
<td>19.18±2.4a^d</td>
<td>13.74±1.31^c</td>
<td>9.66±0.31^d</td>
<td>18.59±0.53^c</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.41±0.04^d</td>
<td>0.54±0.23^d</td>
<td>1.78±0.21^b</td>
<td>1.30±0.41^b</td>
<td>0.23±0.04^f</td>
<td>0.85±0.27^c</td>
<td>1.22±0.16^b</td>
<td>0.24±0.06^f</td>
<td>0.49±0.03^d</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>67.01±1.91^d</td>
<td>99.31±2.19^b</td>
<td>80.68±1.36^e</td>
<td>62.82±1.96^c</td>
<td>31.20±1.25^g</td>
<td>87.73±1.61^b</td>
<td>81.35±0.95^e</td>
<td>37.75±2.01^b</td>
<td>79.33±1.43^f</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>97.13±1.73^d</td>
<td>148.08±1.59^e</td>
<td>116.34±1.43^b</td>
<td>118.24±1.45^b</td>
<td>60.33±1.03^f</td>
<td>101.82±1.26^d</td>
<td>99.45±1.22^d</td>
<td>67.25±1.58^e</td>
<td>96.16±1.98^d</td>
</tr>
</tbody>
</table>

All values were expressed as Mean ± SE. Means with different letters represent significant difference (P<0.05) within the same column; the mean with letter (a) was the highest value.

D0, Control diet; D1-D4, Diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10 g/kg diet, respectively); D5-D8 are diets containing different concentrations of crude FDDP (5, 10, 20, 30 g/kg diet, respectively).

3.12. The effect of FDDP supplementation on antioxidant capacity and associated oxidative stress in liver tissue

All experimental groups had higher liver GSH levels than the D0 control group (Fig. 4a). The D7 group showed the highest significant GSH level at P<0.05. Moreover, all groups had higher SOD activity than the control (D0), except D1, which had lower SOD activity. Among the diet groups supplemented with ethanolic extract (D1, D2 and D3), D4 had the highest significant SOD activity, while D7 had the highest significant SOD specific activity (Fig. 4b). The results indicate that, there was a significant reduction (P<0.05) in the liver TBARS levels of the D3, D4 groups, and D7 groups versus the D0 group. The D3 and D4 groups showed the lowest TBARS levels among other diet groups supplemented with FDDP ethanolic extract (D1, D2 and D3). The D7 group showed the lowest TBARS level among other diet groups supplemented with crude FDDP at P< 0.05 (Fig. 4c). Furthermore, all experimental groups showed a significant reduction in liver NO levels (P< 0.05) except for D2, except for D2, which showed no difference with D0. While, the D1 group showed a significant increase in NO levels compared to all other groups; no differences were detected in NO levels between D4 and D7 among diet groups, supplemented with ethanolic extract or crude FDDP at P< 0.05 (Fig. 4c).
Fig. 4. Alterations in liver antioxidant capacity and associated oxidative stress parameters of different experimental groups

(a) liver GSH levels, (b) liver SOD activities, and (c) liver Oxidative stress biomarkers. All values were expressed as Mean ± SE. Means with different letters represent significant difference (P< 0.05) within the same column; the mean with letter (a) was the highest value. D0, Control diet; D1-D4 are diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/ kg diet, respectively); D5- D8 are diets with different concentrations of crude FDDP (5, 10, 20, 30g/ kg diet, respectively).
3.13. The effect of FDDP supplementation on antioxidant capacity and associated oxidative stress in brain tissue

Results showed a significant increase ($P<0.05$) in brain GSH level and SOD specific activity (Fig. 5a, b) of all experimental groups, compared to D0 (control group). Among the diet groups supplemented with ethanolic extract, the D4 group had the highest GSH level and SOD specific activity. The D7 group had the highest GSH level and SOD specific among other diet groups supplemented with crude FDDP (D5, D6, D8) at $P<0.05$. In all experimental groups (D2, D3, D4, D5, D6, D7, D8), the brain TBARS and NO levels were significantly reduced (Fig. 5c) compared to the D0 control group, except for the D1 group that showed a non-significant change. The D4 and D7 groups showed the lowest TBARS and NO levels among other diet groups supplemented with either FDDP crude or ethanolic extract. Additionally, the results indicate a significant decrease ($P<0.05$) in AChE specific activity in all experimental groups versus the D0 control group (Fig. 5d). Among the diet groups supplemented with either FDDP crude or ethanolic extract, the D4 and D7 groups had the lowest AChE specific activity.
Fig. 5. Alterations in brain antioxidant capacity, associated oxidative stress parameters and AChE activity of different experimental groups

(a) brain GSH levels, (b) brain SOD activities, (c) brain Oxidative stress biomarkers, and (d) brain AChE activities. All values were expressed as Mean ± SE. Means with different letters represent significant difference (P < 0.05) within the same column; the mean with letter (a) was the highest value. D0, Control diet; D1-D4, Diets containing different concentrations of FDDP ethanolic extract (2.5, 5, 7.5, 10g/kg diet, respectively); D5-D8 are diets containing different concentrations of crude FDDP (5, 10, 20, 30g/kg diet, respectively).

DISCUSSION

Date seed is a rich source of a wide variety of nutritive and bioactive compounds, including flavonoids, phenolics that are essential in reducing or controlling the harmful effects of oxidative stress. Besides, date seeds include nutritive compounds such as essential oils, vitamins and minerals (Al-Zubaidy et al., 2016; Theyab et al.)
Effect of Fungi-Degraded Date Pits Supplemented Diet on *Oreochromis niloticus*

*al., 2017*. Owing to its high content of phytonutrients with antioxidant properties, date seeds have some health advantages, viz. reducing lipid oxidation, oxidative stress and free radical damage. Date pits have more protein, fiber and fat than date pulp. Date pits are used to feed fish in the Arabian Gulf (*Belal & Al-Owafeir, 2005*) in addition to their use in feeding sheep and cattle (*Al-Farsi & Lee, 2011*). Carbohydrates found in date pits must be broken down by specific enzymes before used by animals, particularly by fish (*Belal, 2008*). Hydrolysis of cellulose and hemicellulose by xylanases enzymes releasing nutrients bound to these fibers commonly occur in nature (*Leisola et al. (2002)*). Viscosity is reduced and feed utilization is improved, resulting in less waste. Since enzymes in animal feed are expensive, using microorganisms that produce xylanases is a more cost-effective option for degrading dietary fiber. Plant's macronutrients are broken down into organic acid, simple sugars and free amino acids (*Nkhat et al., 2018; Xiang et al., 2019*). In the current study, *Trichoderma reesei* was used, which is the most studied cellulolytic fungus (*Foreman et al. (2003)*) to prepare FDDP. *Trichoderma reesei* degradation process was demonstrated in the present study both physically and chemically.

The electron microscope picture of freeze-dried date pits before degradation confirmed tightly packed structures and low nanosized-pore volume in accordance with a previous study (*Hossain et al., 2014*). While, the different *Trichoderma reesi* colonies present indicate that *Trichoderma reesei* grew on date pits material by reducing stickiness and breaking down date pits particles. This raise in date pits particles surface areas that are exposed to digestive enzymes in the fish gut as mentioned by *Belal (2008)*.

In our study, after two weeks of inoculation process and in agreement with *Belal (2008)*, FDDP crude protein, crude fiber, ash, moisture, NDF and hemicellulose degradation increased, while ADF and cellulose decreased compared to DDP. It indicates chemical changes during degradation; decrease in total carbohydrate, increase in free sugars and digestible carbohydrate content were also observed. These results surpass all previous studies on date pits (*Bouhlali et al., 2016; El-Rahman & Al-Mulhem, 2017; Metoui et al., 2019*). This may be due to the liberation of nutrients blocked within date pits fibers that would have led to more digestible nutrients from the DDP. *Leisola et al. (2002)* postulated that, xylanases (produced by *Trichoderma reesei*) liberate nutrients either by hydrolysis of non-degradable cellulose or hemicelluloses. Two weeks after inoculation, xylanase and carboxymethyl cellulase activities increased twice, confirming the degradation process in agreement with *Zhang et al. (2012)*.

According to *Rahman et al. (2007)*, date pits contain varying amounts of macro and trace elements depending on date cultivar, climate, irrigation water, and
fertilizers used to harvest them. Fortification of food products with FDDP is possible due to the element and mineral contents. Adeosun and Bouhlali (2016) reported higher potassium, phosphorous, magnesium, and zinc content than reported in our study.

Since polyphenols solubility varies depending on solvent polarity (Maqsood et al., 2020). In the present study, we prepared aqueous and 70% ethanolic extracts of DDP and FDDP. The ethanolic extract had the highest phenolic and flavonoid contents. Our results were higher than those of Hussain et al. (2020) who studied total phenolic contents in six varieties of date palm pits extracted in different organic solvents.

The extract IC<sub>50</sub> is inversely associated with its richness in antioxidant compounds, in other words, lower IC<sub>50</sub> value indicates a higher antioxidant activity (Al-Farsi & Lee, 2008). Our results exhibited higher scavenging activity of FDDP and DDP than values recorded in previous studies (Al-Farsi & Lee, 2008; Thouri et al., 2017). Interestingly, our findings revealed that, the FDDP aqueous extract has the highest NO scavenging activity, followed by FDDP ethanolic extract. The lower IC<sub>50</sub> of our extracts indicates greater antioxidant activity. Date seeds are able to quench NO, thus they are very important in avoiding the negative effects of excessive NO production (Bouhlali et al., 2020).

The highest IC<sub>50</sub> value was obtained for FDDP ethanolic extract and it corresponded to be the safest, compared to other extracts, while DDP aqueous extract had the lowest IC<sub>50</sub> value, making it the most toxic to human WBCs. Similarly, Dawood et al. (2020) found that, adding fermented date seed meal to the diet of the Nile tilapia improved their hematological parameters (RBCs, WBCs and HB). This explains their immunostimulatory and anti-infection properties (Apeh et al., 2014).

The HPLC chromatogram of polyphenolic content of DDP and FDDP ethanolic extracts revealed that, the extracts displayed phenolic profiles with great differences, with the highest concentrations of hydroxy benzoic acid (p-hydroxy benzoic acid) and syringic acid and two cinnamic acid derivatives (caffeic and sinapic acids) that were found in FDDP ethanolic extract. Moreover, apigenin-7-O-Glucoside, luteolin, naringenin and chrysin were found in higher concentration in FDDP ethanolic extract, compared to DDP. In accordance with Ahmed (2017) and Eid et al. (2014), the most abundant phenolic compounds in (ajwa) date seeds are p-coumaric acid, ferulic acid, gallic acid, caffeic acid, p-hydroxybenzoic acid, and syringic acid, with luteolin and apigenin forming the most abundant flavonoids.
The beneficial effects of using dates as a feed additive on fish performance including the Nile tilapia (Belal, 2008; Azaza et al., 2009; Gaber et al., 2014), the African cat fish (Sotolu et al., 2014) and common carp (Hoseinifar et al., 2017) were widely documented. However, a few studies such as Belal and Al-Owafeir (2005) and El-Sayed et al. (2006) found that, date seed supplementation reduced tilapia growth and body fat. This may be due to the presence of high levels of crude fiber and non-digestible carbohydrates in date pits. Contrarily, Belal (2008) observed that, the weight gain was increased after the administration of FDDP as a feed supplement at concentrations of 150, 300, 450 & 600mg/ kg. This indicates that FDDP affected positively fish growth and attributed the increment in fish growth to the increase of digestible carbohydrates contents and the liberated nutrients and also to the high levels of free sugar mannose as a result of Trichoderma reesei fungi action.

Remarkably, in our study we found that, diets supplemented with D4 (1% ethanolic FDDP extract) and D7 (2% Crude FDDP) groups grew faster than all other tested groups. In addition, they significantly increased PER and SGR values. The improvement in nutrient utilization may be attributed to the increment of polyphenolic compounds level in FDDP, which act as natural antioxidants. Furthermore, fermentation process induces degrading of proteins into smaller peptides, which are more efficiently absorbed by intestinal cells (Dawood et al., 2020).

We noticed the increase in HSI, VSI, and K values at D4 and D7 diets in accordance with Kamali-Sanzighi et al. (2019) who found that, adding 10% date seeds powder to a common carp diet increases growth but adding more date pits may reduce fish growth due to high fiber content and unsaturated fatty acids such as oleic acid (Sotolu et al., 2014). In addition, adding FDDP in both forms significantly raises K values, compared to the control group, indicating improved fish welfare in accordance with the findings of Hossain et al. (2014) who attributed this improvement to the presence of beneficial bioactive materials in date pits.

After 10 weeks of feeding, our results revealed the dietary differences in fish biochemical compositions, where fish fed on FDDP, either in crude or extract forms, showed higher protein content, indicating the improvement in feed utilization through altering protein synthesis and muscle deposition rate (Abdel-Tawwab et al., 2008). The anti-bacterial activity of FDDP extract or crude reduces pathogens in fish intestine, allowing more nutrients to be absorbed and converted into body mass (Assem et al., 2014).
Furthermore, fish welfare was confirmed through biochemical analyses, where the highest hemoglobin content was found in D4 and D7 groups, indicating increased oxygen supply and consequently increased health effects on fish (Vahedi et al., 2017). Although the D4 group showed normoglycemia, the addition of FDDP crude or ethanolic extract to fish feed diets resulted in significant increase in blood sugar concentration, which concurs with the findings of both Masoudi et al. (2011) and Kamali-Sanzighi et al. (2019) who stated that, glucose was highly accentuated when fibrous diets were added to the food in common carp and broilers, respectively, as fibrous material helped to increase the activity of gluconeogenesis.

Further, the presence of polyphenols as naringenin, chrysin and luteolin exhibits potent anti-hyperuricemic and anti-inflammatory effects (Uckun et al., 2020), which made FDDP supplementation in fish diets beneficial. Date seeds also protect against hepatotoxicity and renal toxicity by detoxifying free radicals and inhaling lipid peroxidation and nitric oxides (Mousalmy & Hussein, 2016; Ahmed, 2017).

Liver enzymes are important indicators of liver function where any increase in these enzymes may indicate liver damage (Wang et al., 2001). In agreement with previous study, adding FDDP ethanolic extract (at concentration of 10g/ kg diet (D4 group) and FDDP crude (at concentration of 20g/ kg diet (D7 group) to fish feed diets for 45 days reduced liver enzyme activity (Kamali-Sanzighi et al., 2019). Date pits’ hepatoprotective effect was linked to polyphenolic compounds and minerals like zinc, copper, potassium and manganese (Saryono & Proverawati, 2019).

Notably, supplementing fish diets with FDDP increased GSH levels in both liver and brain homogenates, especially at D4 and D7 concentrations. Antioxidant enzyme as SOD act as a defense mechanism against ROS as SOD decomposes superoxide radical (O$_2^-$) to produce H$_2$O$_2$. SOD activity in liver and brain increased by increasing of concentration of FDDP supplementation either crude or ethanolic extract in fish diet especially in D4 and D7 diets. Besides, FDDP’s ability to reduce accumulation of free radicals caused by lipid peroxidation or stresses on fish was explained. Similarly, Alyileili et al. (2020) found that supplementing broiler feed with 10% FDDP increased antioxidant enzyme syntheses such as SOD, GPX.

In our study, FDDP crude and ethanolic extracts reduced TBARS and NO levels in liver and brain homogenates, with the lowest values at D4 and D7 diets indicating that FDDP inhibits lipid peroxidation and NO production by inhibiting NOS activity. Likewise, Maqsood et al. (2020) and Hoseinifar et al. (2017)
stated that date seeds extracts possess protective and anti-inflammatory effects. This is related to their activity in enhancing activities of antioxidants as SOD, GST, and reduction in TBARS level, thus thy acted as good antioxidants.

In agreement with Sumanth and Mamatha (2014) who found that Phoenix dactylifera enhanced learning and memory in the Morris water maze and suggested its mechanism by decreasing acetylcholine esterase activity leading to accumulation of acetylcholine and thus enhancing memory. In our work, we found that either supplemented crude or ethanolic extract of FDDP especially at concentration of 1% ethanolic extract (D4) and 2% crude extract (D7) decreased AChE specific activity. In fact, dates are rich in polyphenolic compounds that have anticholinesterase activities, so FDDP can act as cholinergic system activator or free radical scavenger (Tumiatti et al. (2008)).

We had screened various concentrations of both FDDP crude and ethanolic extract to identify which ones were most comparable to the control group and had the most effective biological and physiological impacts on fish health. Our results indicated that they were (D4 and D7) groups in FDDP ethanolic and crude; respectively. We intended to use these concentrations in our subsequent researches as the most potent FDDP supplements for fish diets.

**CONCLUSION**

The present study revealed that utilization of fungi degraded date pits as a feed additive is capable of improving fish growth performance and nutrient utilization as well as ensuring production of healthy fish on sustainable basis. Supplementation of 1% ethanolic FDDP extract and/or 2% crude FDDP in tilapia diet enhanced the growth performances by improving final weight, feeding efficiency and specific growth rate. This study further revealed that FDDP incorporated diet did not show any adverse effect in body composition and hematological profile in the tilapia but also enhanced oxidant and antioxidant status. We recommend the use of FDDP as an excellent feed additive in Nile tilapia diets.

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Supplementary Figure (1): Typical chromatograms for determination of bioactive compounds in DDP and FDDP ethanolic extracts.

(A) Mixed standards; (B) HPLC chromatogram of DDP ethanolic extract; (C) HPLC chromatogram of FDDP ethanolic extract.