Whey protein concentrate powder as a growth promoter and an immunostimulant agent for Oreochromis niloticus (Linnaeus, 1758) fingerlings

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INTRODUCTION

Nowadays, aquaculture is considered a fast-growing industry that supports the world with a relatively cheap protein source. During the last few decades, aquaculture production has been doubled (FAO, 2020). According to the latest aquaculture statistics in Egypt, aquaculture is currently the largest single source of fish supply accounting for almost 80.5% of the total fish production of the country with an estimation of over 99% is produced from private farms (GAFRD, 2019). Globally, tilapias are considered a highly important species in aquaculture, as they ranked second to carps. The Nile tilapia (Oreochromis niloticus), for instance, is the most widely spread fish in aquaculture with an annual growth rate of 8.0% (FAO, 2020). The high popularity of the tilapia in Egypt and the world may be due to their ability to feed on a wide range of feeds, their fast growth, temperate nature, less requirement of the alien species and low price of the feedstuff (FAO, 2020). However, the high demand for Nile tilapia may lead to the decrease in fish production and health issues due to the susceptibility to bacterial and viral infections (Folayan et al., 2020). The Nile tilapia juveniles are highly susceptible to bacterial and viral infections (Garritano et al., 2011; Folayan et al., 2017). Moreover, increasing production costs have led to searching for cheaper and more effective feed additives to improve the growth performance of aquatic animals (Abd El-Magid and Gamal El-Din, 2016; Al-Nasser and El-Baker, 2017; Gubareva et al., 2018). Due to their nutritive value and functionality, whey proteins have been used in many food applications. The efficacy of adding different levels (0, 5, 10 and 20 g kg⁻¹ diet) of whey protein concentrate powder (WPCP) was addressed on the growth performance, feed utilization, and the immune responses of Oreochromis niloticus fingerlings in the current study for 8 weeks. A significant increase was observed in the growth performance (final weight, total weight gain, average daily gain, relative growth rate, and specific growth rate), the feed utilization (feed conversion ratio, protein efficiency ratio, protein productive value, and energy utilization), and the whole-body composition (dry matter, and crude protein) parameters. These levels also significantly improved both the hematological parameters (hemoglobin, red blood cells, packed cell volume, and platelets), and the immune responses indicators (white blood cells, lymphocytes, and serum immunoglobulin M) of the O. niloticus fingerlings. Partially, in all tested parameters, the addition of the high-level 20g WPCP kg⁻¹ diet showed a remarkable superiority among other levels. Remarkably, the beneficial use of WPCP at the level of 20 g kg⁻¹ diet was proved as a promising growth promoter and immune stimulant agent for O. niloticus fingerlings, and may in return, increase fish productivity, health, and profitability in fish farms.
growth rates, high conversion ratio, and potential to reproduce in captivity (El-Sayed, 2006).

The successful aquaculture depends on a nutritionally balanced diet and a low cost of production. (Yousefi et al., 2018). Feed represents around 50 – 80% of production cost in aquaculture, and nutrition is one of the critical factors. To overcome escalating costs, feed companies have turned to the application of functional feed additives, which led to the growth rate and immune response improvements and induced the physiological functions and health performance of fish more than the normal feed additives (Alemayehu et al., 2018). According to the different benefits of feed additives, as appetite increaser, digestion regulators, and supporting effects to increase growth performance of the fish, the use of additives in feeds has recently increased. Thus, the purpose of the use of feed additives in aquaculture is to increase efficiency, quality, and profits (Ebru & Cengiz, 2016). In this regard, a lot of attention in this field has been given to the use of different types of feed additives for fish, such as probiotics (Mehrim, 2009, Abdelhamid et al., 2013; Mehrim et al., 2015, 2017, 2019a). Prebiotics were also used as feed additives (Mazurkiewicz et al., 2008; Mehrim et al., 2013). Additionally, medicine herbs (Asimi and Sahu, 2013; Khalil et al., 2014, 2015; Mehrim et al., 2014, 2019b) and exogenous enzymes (Ayhan et al., 2018; Zheng et al., 2019) were determined. Among other feed additives for fish, the organic acids and their salts were reported (Hossain et al., 2007; Khajepour & Hosseini, 2012; Khaled, 2015) whereas some researchers mentioned the use of minerals (Morken et al., 2011; Mehrim, 2012, 2014).

Whey protein (WP) is widely considered the highest quality natural protein (Wolfe, 2000). It can be utilized well when fed in a variety of forms, including liquid whey, condensed whey, dried whey, or dried whey products. WP has a high concentration of branched-chain amino acids (BCAAs) – leucine, isoleucine, and valine, which are important factors with respect to tissue growth and repair, and they play an important role during the translation-initiation pathway of protein synthesis (Anthony et al., 2001). The components of WP include β-lactoglobulin (β-LG), α-lactalbumin (α-LA), bovine serum albumin (BSA), lactoferrin, immunoglobulins, lactoperoxidase enzymes, glycomacropeptides, lactose, and minerals, as well as whey derived from buttermilk that contains the lipid sphingomyelin (Walzem et al., 2002). WP is also rich in the sulfur-containing amino acids of cysteine and methionine, which possess an immune function through the intracellular conversion to glutathione. It also contains bioactive substances, such as hormones, growth factors, and cytokines, which can have an important physiological role that are able to regulate the cell growth (Sukkar & Bounous, 2004). It was reported that the inclusion of WP in the diets of the experimental animal models like broiler might enhance growth performance and improve the growth of the intestinal mucosa layer and caecal microflora community (Alloui & Szczurek, 2017; Pineda-Quiroga et al., 2018). Regarding the fish, both of Abdel-Tawwab and Abbass (2016) and Amer et al. (2019) stated that the high substitution levels of WP were not
recommended in the diets of the *O. niloticus* fingerlings. To the current researchers’ knowledge, little attention has been given with respect to using WP as a feed supplement for different animal models such as weanling pigs (*Grinstead et al., 2000*); rats (*Eason et al., 2004*); broiler chickens (*Afkhami et al., 2020*), and Japanese quail (*Jazi et al., 2020*). However, there is still limited information on the influence of WP as an additive in fish diets. Thus, the present study was designed to evaluate the effects of different levels (0, 5, 10, and 20 g kg\(^{-1}\) diet) of whey protein concentrate powder (WPCP) on growth performance, feed utilization, the chemical composition of the fish body, and the immune responses of *O. niloticus* fingerlings reared in glass aquaria for 8 weeks.

**MATERIALS AND METHODS**

1. **The experimental procedures**
   
   This experiment was conducted in Fish Research Laboratory, Faculty of Agriculture, Mansoura University, Al-Mansoura, Egypt. The *O. niloticus* fingerlings (n = 84; average initial body weight of each was 16.75 ± 0.14 g) were adapted with the experimental conditions for 10 days, and were fed the control diet twice daily by hand (9.00 a.m. and 14.00 p.m.). After the adaptation period, fish were randomly distributed into four treatments (three replicates/treatment). Fish were stocked at twelve glass aquaria (90×40×35 cm; total volume of 108 L). Each aquarium was stocked with seven fish and supplied with an air electric compressor for water aeration. To remove the wastes in each aquarium, water was partially changed every day, and dechlorinated tap water was added. Water temperature was measured via a thermometer, the pH values were determined using Jenway Ltd., Model 350-pH-meter, and dissolved oxygen (DO) was tested using Jenway Ltd., Model 970-DO-meter. These water quality parameters were measured twice a week, where mean values of water temperature ranged between 25.6 and 27.8°C, and the pH values were 6.70 – 7.90, whereas the recorded DO ranged from 5.50 – 6.70 mg L\(^{-1}\). Notably, these water quality criteria are acceptable for rearing the *O. niloticus* fingerlings according to *Boyd and Tucker (2014)*.

2. **Experimental diet preparation**
   
   The ingredients of the basal diet (BD) were bought from the local market and were homogeneously mixed with the corn oil. The ingredients of BD and chemical analysis were carried out according to *AOAC (2004)* and results are shown in Table (1). The WPCP was carefully added to the BD at levels of 0 (T\(_0\)), 5 (T\(_5\)), 10 (T\(_{10}\)), and 20 (T\(_{20}\)) g kg\(^{-1}\) diet. The experimental diets were introduced to the fish by hand twice daily at 9.00 a.m. and 15.00 p.m. at 5% of the total fish biomass. In each aquarium, the fish were weighed every two weeks by a digital scale (accurate to ± 0.01 g) to adjust their feed quantity according to the real body weight changes.
Table 1: Ingredients and proximate chemical analysis (% on dry matter basis) of the experimental basal diet

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish meal (72% CP)</td>
<td>8</td>
</tr>
<tr>
<td>Corn gluten (60% CP)</td>
<td>15</td>
</tr>
<tr>
<td>Soybean meal (44% CP)</td>
<td>28</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>20</td>
</tr>
<tr>
<td>Yellow corn</td>
<td>20</td>
</tr>
<tr>
<td>Molasses</td>
<td>3</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>5</td>
</tr>
<tr>
<td>Vitamins and minerals premix</td>
<td>1</td>
</tr>
</tbody>
</table>

**Nutrient composition**

<table>
<thead>
<tr>
<th>Nutrient composition</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter (DM, %)</td>
<td>93.53</td>
</tr>
<tr>
<td>Crude protein (CP, %)</td>
<td>30.91</td>
</tr>
<tr>
<td>Ether extract (EE, %)</td>
<td>7.23</td>
</tr>
<tr>
<td>Ash (%)</td>
<td>5.64</td>
</tr>
<tr>
<td>Total carbohydrates (%)</td>
<td>56.22</td>
</tr>
<tr>
<td>Gross energy (GE, MJ 100 g⁻¹ DM)²</td>
<td>2004.3</td>
</tr>
</tbody>
</table>

¹ Each 3 Kg premix contains: Vit. A, 12000,000 IU; Vit. D₃, 3000,000 IU; Vit. E, 10,000 mg; Vit. K₃, 3000 mg; Vit. B₁₂, 200 mg; Vit. B₆, 3000 mg; Vit. B₁₂, 15 mg; Biotin, 50 mg; Folic acid, 1000 mg; Nicotinic acid, 35000 mg; Pantothenic acid, 10,000 mg; Mn, 80g; Cu, 35 g; I, 1g; Co, 0.15g and Se, 0.3g.

² GE (MJ 100 g⁻¹ DM) = (CP x 23.64) + (EE x 39.54) + (Total carbohydrates x 17.57) calculated according to NRC (1994).

3. Fish sampling and the tested parameters

3.1. Growth performance

At the end of the experiment, fish were weighed to calculate the growth performance parameters following the method of Lovell (2001) as follows;

Total weight gain (TWG, g) = FW (g) – IW (g).

Average daily gain (ADG, g fish⁻¹ day⁻¹) = \( \frac{TWG}{T} \).

Relative growth rate (RGR, %) = \( \frac{TWG}{IW} \times 100 \).

Specific growth rate (SGR, % day⁻¹) = \( \left[ \frac{(\ln FW - \ln IW)}{T} \right] \times 100 \).

Condition factor (K, %) = \( \left( \frac{FW}{TL^3} \right) \times 100 \).

Survival rate (SR, %) = \( \left( \frac{\text{No. of fish at the end of the study}}{\text{No. of fish initially stocked}} \right) \times 100 \).

Where:

FW: Final weight (g); IW: Initial weight (g); TL: Total length (cm); T: The experimental period (day).
3.2. Feed utilization parameters

Feed intake (FI, g) was recorded and feed utilization parameters were calculated according to Lovell (2001) following the succeeding equations:

Feed conversion ratio (FCR) = \( \frac{\text{FI}}{\text{TWG}} \).

Protein efficiency ratio (PER) = \( \frac{\text{protein intake}}{\text{protein intake}} \).

Protein productive value (PPV, %) = \( \left( \frac{\text{Protein stored in the fish body}}{\text{Protein intake}} \right) \times 100 \).

Energy utilization (EU, %) = \( \left( \frac{\text{Energy stored in the fish body}}{\text{Energy intake}} \right) \times 100 \).

3.3. The chemical composition of fish body

At the beginning of the experiment, twenty fish were kept as a pooled sample. At the end of the experiment, five fish in each treatment were collected and kept frozen (-20 °C) till the proximate analysis of the whole fish body was done according to AOAC (2004). While, the energy content of the fish body was calculated according to NRC (1994).

3.4. Hematological parameters

At the end of the experiment, nine fish in each treatment were randomly taken and anesthetized with three drops of commercial clove oil extract dissolved in 10 L of tap water to get the blood samples. Blood was carefully collected from the fish caudal peduncle of all treatments. Adequate amounts of the whole blood (5 mL at each collection) were received in small plastic vials containing heparin to determine the hematological parameters. Hemoglobin (Hb) was measured using commercial colorimetric kits (Diamond Diagnostic, Egypt). Total red blood cells (RBCs \( \times 10^6 \text{ mm}^{-3} \)), blood platelets (\( \times 10^3 \text{ mm}^{-3} \)), total white blood cells (WBCs \( \times 10^3 \text{ mm}^{-3} \)), and lymphocytes were counted according to Dacie and Lewis (1995) on an Ao Bright – Line Hämocytometer model (Neubauer improved, Precicolor HBG, Germany). However, the packed cell volume (PCV %) was estimated according to Stoskopf (1993).

3.5. Serum immunoglobulin M (IgM) measurement

To obtain the blood serum, other blood samples (5 mL at each collection) were collected in dried plastic tubes and centrifuged for 20 min at 3500 rpm. Serum samples
were kept in a deep freezer (−20 °C) until IgM (mg dL⁻¹) analysis was carried out. Serum IgM was estimated using ELISA test kits catalog No. MBS282651 (sensitivity; 1 μg mL⁻¹; MyBioSource Company, San Diego, USA).

4. Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using the SAS® software for windows (SAS, 2006) to detect the overall effects of treatments (T₀-T₂₀), followed by the comparison of means using Tukey’s post hoc significant test. The statements of significance between the means were based on a probability level of \( P \leq 0.05 \). All ratios and percentages were arcsine-transformed prior to statistical analyses.

RESULTS

1. Fish growth performance

The results presented in Table (2) reveal that, the \( O. \) niloticus fingerlings in T₂₀ significantly increased all the growth performance parameters among all the experimental treatments (\( P \leq 0.05 \)). While the same treatment (T₂₀) led to an insignificant increase of SR and K-factor among to the other treatments (\( P \geq 0.05 \)).

<table>
<thead>
<tr>
<th>Traits</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₀</td>
</tr>
<tr>
<td>Final weight (g)</td>
<td>39.64±0.53ᵇ</td>
</tr>
<tr>
<td>Total weight gain (g)</td>
<td>22.89±0.53ᵇ</td>
</tr>
<tr>
<td>Average daily gain (g fish⁻¹ day⁻¹)</td>
<td>0.38±0.01ᵇ</td>
</tr>
<tr>
<td>Relative growth rate (%)</td>
<td>136.7±3.19ᵇ</td>
</tr>
<tr>
<td>Specific growth rate (% day⁻¹)</td>
<td>1.44±0.02ᵇ</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>90.47±4.76ᵇ</td>
</tr>
<tr>
<td>Condition factor (K, %)</td>
<td>1.74±0.05ᵇ</td>
</tr>
</tbody>
</table>

Different small letters in the same row indicate significant differences among treatments (\( P \leq 0.05 \)).

2. Feed efficiency

The feed efficiency parameters of the \( O. \) niloticus fingerlings fed WPCP-supplemented or non-supplemented diet (T₀) are showed in Table (3). Fish fed supplemented diet with different levels of WPCP, especially at the high level (T₂₀) significantly increased the feed efficiency parameters (PER, PPV, and EU), and significantly improved the FCR compared to the control group. While, these enhanced feed efficiency parameters are dosage-dependent (\( P \leq 0.05 \)).
Whey protein as a growth promoter and an immunostimulant agent for *O. niloticus*

Table 3: Effect of different levels of dietary whey protein concentrate powder on feed efficiency parameters of *Oreochromis niloticus*

<table>
<thead>
<tr>
<th>Traits</th>
<th>Treatments</th>
<th>T₀</th>
<th>T₅</th>
<th>T₁₀</th>
<th>T₂₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake (g)</td>
<td></td>
<td>66.87±3.31</td>
<td>67.50±1.40</td>
<td>66.09±3.79</td>
<td>72.04±4.61</td>
</tr>
<tr>
<td>Feed conversion ratio</td>
<td>2.92±0.16ᵃ</td>
<td>2.51±0.14ᵇ</td>
<td>2.49±0.22ᵃᵇ</td>
<td>2.12±0.13ᵇ</td>
<td></td>
</tr>
<tr>
<td>Protein efficiency ratio</td>
<td>1.11±0.06ᵇ</td>
<td>1.29±0.07ᵇ</td>
<td>1.32±0.12ᵃᵇ</td>
<td>1.54±0.10ᵃ</td>
<td></td>
</tr>
<tr>
<td>Protein productive value (%)</td>
<td>17.44±2.14ᵇ</td>
<td>21.77±1.43ᵃᵇ</td>
<td>20.05±2.39ᵃᵇ</td>
<td>28.21±1.82ᵃ</td>
<td></td>
</tr>
<tr>
<td>Energy utilization (%)</td>
<td>11.15±0.46ᵇ</td>
<td>13.82±0.73ᵃᵇ</td>
<td>11.35±1.27ᵇ</td>
<td>16.24±1.27ᵃ</td>
<td></td>
</tr>
</tbody>
</table>

Different small letters in the same row indicate significant differences among treatments (*P* ≤ 0.05).

3. The chemical composition of the fish

Fish fed a high level of WPCP (T₂₀) significantly increased (*P* ≤ 0.05) both dry matter and crude protein compared to T₀ (Table 4). However, fish in T₅ displayed significantly increased crude fat and energy content among all treatments. Meanwhile, the control treatment (T₀) showed significantly increased ash content among all treatments (*P* ≤ 0.05).

Table 4: Effect of different levels of dietary whey protein concentrate powder on the chemical composition of the fish body of *Oreochromis niloticus*

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Dry matter (%)</th>
<th>Ash (%)</th>
<th>Fat (%)</th>
<th>Protein (%)</th>
<th>Energy content (MJ 100 g⁻¹ DM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₀</td>
<td>22.97±1.06ᵇ</td>
<td>18.46±0.34ᵃ</td>
<td>18.68±0.26ᵇ</td>
<td>62.67±0.08ᵇ</td>
<td>2227±12.00ᶜ</td>
</tr>
<tr>
<td>T₅</td>
<td>24.65±0.57ᵃᵇ</td>
<td>15.18±0.09ᶜ</td>
<td>22.81±0.40ᵃ</td>
<td>62.00±0.36ᵇ</td>
<td>2367±7.68ᵃ</td>
</tr>
<tr>
<td>T₁₀</td>
<td>21.92±0.40ᶜ</td>
<td>16.33±0.40ᵇ</td>
<td>19.71±0.62ᵇ</td>
<td>63.95±0.45ᵃ</td>
<td>2291±17.57ᵇ</td>
</tr>
<tr>
<td>T₂₀</td>
<td>25.84±0.58ᵃᵇ</td>
<td>16.27±0.29ᵇ</td>
<td>18.84±0.13ᵇ</td>
<td>64.89±0.36ᵃ</td>
<td>2278±6.33ᵇ</td>
</tr>
</tbody>
</table>

Different small letters in the same column indicate significant differences among treatments (*P* ≤ 0.05).

Energy content (MJ100 g⁻¹ DM) in the whole fish body was calculated according to NRC (1994), being 23.64 and 39.54 MJ g⁻¹ for protein and fat, respectively.

4. Hematological parameters

*O. niloticus* fingerlings fed high levels of WPCP (T₁₀ and T₂₀) significantly increased both RBCs and Hb compared to those in T₅ or T₀ (Fig. 1), where *P* ≤ 0.05. However, T₂₀ showed a significant increase of PCV and blood platelets among all treatments (*P* ≤ 0.05).
Fig. 1: Effect of different levels of dietary whey protein concentrate powder on hematological parameters (A) Red blood cells (RBCs), (B) Hemoglobin (Hb), (C) Packed cell volume (PCV), and (D) Platelets of *Oreochromis niloticus*. Vertical bars indicate the standard error. Small letters indicate significant differences among treatments (*P* ≤ 0.05).

5. Immune indicators measurements

Immune responses parameters (as WBCs, lymphocytes, and IgM) significantly increased with the increasing levels of WPCP (Fig. 2), where *P* ≤ 0.05. The T20 recorded the highest values of immune responses parameters compared to other treatments.
**DISCUSSION**

In aquaculture, the enhancement of the growth performance of the fish is one of the major crucial goals (Dada, 2015). The WP plays an important role in improving growth performance, survival rate, immune responses, and gut health (Amer et al., 2019). In the current study, the WPCP at high levels in T₁₀ and T₂₀, significantly increased growth performance and feed efficiency parameters of the *O. niloticus* fingerlings among other treatments. These positive effects of WPCP could be attributed to the high content of essential amino acids in the WPCP, which are principally required for protein synthesis.

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**Fig. 2:** Effect of different levels of dietary whey protein concentrate powder on immune indicators parameters (A) White blood cells (WBCs), (B) Lymphocytes, and (C) Serum immunoglobulin M (IgM) of *Oreochromis niloticus*. Vertical bars indicate the standard error. Small letters indicate significant differences among treatments (*P* ≤ 0.05).
and energy (Yoshizawa, 2004). This may also be related to the role of WPCP for increasing the growth hormone, improving the gut health, increasing the intestinal absorptive surface, and the number of goblet cells that were detected by Amer et al. (2019). Similarly, with the current findings, Afkhami et al. (2020) recently suggested the use of WP for improving the growth performance of broiler chickens. The previous authors also reported that, the WP may provide minerals (calcium, and phosphorous) for bone mineralization, and may hence, help to improve growth performance.

In this context, Szczurek et al. (2013) also stated that dietary with WP improves growth through balancing amino acids and carbohydrates in broiler chickens. In addition, Jazi et al. (2020) concluded that, the WP has beneficial roles to enhance the product performance, gut functionality, and antioxidant capacity of Japanese quail, which might be potentially related to the lactose fermentation and short-chain fatty acids (SCFAs) production, which favor beneficial bacteria such as lactobacillus and improve intestinal integrity of WP. Evidently, an improvement in bird’s performance fed WP may also be explained by highly digestible protein and fat and the presence of water-soluble vitamins in WP (Kermanshahi & Rostami, 2006). Similarly, Pineda-Quiroga et al. (2018) showed that the addition of WP to broiler diets increased average daily gain and body weight during the starter, grower, and the entire feeding periods compared to the control group. Inversely, with the current findings, Abdel-Tawwab and Abbass (2016) found no significant effect of substitution of fishmeal with dried whey meal by 25, 50, 75, or 100% on the growth performance, nutrient utilization, and the whole body composition of O. niloticus fingerlings, except for the lipid content of the fish body, which increased in 100% fishmeal replacement compared to the 0%. Yet, Amer et al. (2019) concluded that whey protein concentrate (WPC) could replace the fishmeal in the O. niloticus fingerlings diets up to 27.7%, to improve the total weight, the gut health, and immune status of fish. They also recommended that high inclusion levels of WPC were not recommended in fish diets.

Regarding the proximate composition of whole fish, the results showed that the body dry matter (DM) and crude protein (CP) contents were significantly increased by increasing levels of dietary WPCP (T20) among all treatments. These findings are mainly related to the positive effects of dietary WPCP on growth performance and feed utilization in the present study. Additionally, previous findings also coincide with those related to WPCP which proved to have an essential role in carbohydrate metabolism by supplying the required energy (Morifuji et al., 2005). WPCP also contains a high number of essential amino acids that play an important role in protein synthesis and carbohydrate metabolism required for supplying energy (Yoshizawa, 2004). Traces of lactic acid and citric acid (as organic acids) were also found in WP (Tsakali et al., 2010). In addition, Zemel (2004) and Smilowitz et al. (2005) reported that, the WP is a rich source of BCAAs (leucine, isoleucine, and valine); playing a role as metabolic regulators in protein and glucose homeostasis and in lipid metabolism. Similarly, with the obtained findings
Whey protein as a growth promoter and an immunostimulant agent for *O. niloticus*

herein, the researchers have found that the increase of WPCP addition level led to a linear increase of DM degradability and a quadratic response of fiber degradability (El-Shewy, 2016). In this respect, Barrows and Frost (2014) reported that the digestibility of whey DM (98%), fat (95%), and CP (98%) were high. Moreover, they found that digestibility of all minerals in whey was high, with 91%, 97%, 70%, and 100% for phosphorus, potassium, sulfur, and zinc, respectively.

Feed containing functional feed additives not only promote the growth and health of tilapia, but also improve their immune systems, and induce physiological benefits beyond traditional feeds (Alemayehu et al., 2018). WPCP significantly develops the immune status more than casein or soy protein-inclosing diets (Tavares & Malcata, 2013). The significantly positive effects of dietary WPCP on the hematological (Hb, RBCs, PCV, and platelets) and immune responses (WBCs, lymphocytes, serum IgM) parameters of *O. niloticus* fingerlings, especially T10 and T20, were recorded in the present study. These effects are also confirmed by Amer et al. (2019) who recorded an improved IgM, nitric oxide and an increase in the lysozyme activity of *O. niloticus* fed WPCP. The current findings explain the role of WPCP in potentiating the innate immunity (Rutherford-Markwick et al., 2005; Galeotti et al., 2013), and stimulating the immune system by ingestion of α-LA, and β-LG the bioactive compounds of WPCP (Groleau et al., 2003; Expósito & Recio, 2006). Moreover, the present results proved the effect of WPCP in improving the humoral immunity related to the WPCP inclusion of the BSA, immunoglobulins (Igs), lactoferrin, and glycomacropeptide (Wong et al., 1998; Low et al., 2001). Furthermore, β-LG; the main protein in WP, is considered as a precursor of bioactive peptides that carries immunomodulatory function (Gauthier et al., 2006). WPCP is also considered as an immune-potentiating dietary protein supplement due to its high levels of sulfur-containing amino acids (Low et al., 2003; Knowles & Gill, 2004). Moreover, some WP-derived peptides have favorable health effects as antimicrobial properties, and antioxidant activities, and also improve mineral absorption, and consequently, have immune-modulatory effects (Pihlanto, 2006; Udenigwe & Aluko, 2012). The mechanisms of the physiological roles of these bioactive peptides involve action upon certain receptors, enzyme inhibitors, intestinal absorption regulation, and display antioxidant or antimicrobial activities (Tavares & Malcata, 2013).

WPCP may have a significant effect on immune function as a rich source of Igs (Bell, 2000). Finally, the superiority of high level of dietary WPCP (T20) among different levels on all the tested parameters of WPCP-treated fish may be seriously related to the high biological value of WP as it contains wide range of bioactive components, such as α-LA, β-LG, BSA, Igs, lactoferrin, and glycomacropeptide, besides it contains the high amounts of BCAAs. It is worth noting that, isolated whey peptides provide the following benefits; increase release of insulin-like growth factor, and improve overall endocrine hormone response (Akhavan et al., 2010; Gaudel et al., 2013). Moreover, they increase nitrogen utilization and retention (Blome et al., 2003; Saito, 2008), increase intracellular
glutathione, act as anti-aging antioxidants (Xu et al., 2011; Athira et al., 2013), improve immune function (Jensen et al., 2012) and improve gastrointestinal health as well (West et al., 2012). In addition, they play a crucial role in increasing the rate of muscle growth (Walrand et al., 2011; Coker et al., 2012).

**CONCLUSION**

The current findings confirmed the beneficial use of dietary addition of WPCP as a promising growth promoter or/and an immune stimulant agent, especially at a high-level (20g kg\(^{-1}\) diet (T\(_{20}\)), for *O. niloticus* fingerlings. Nevertheless, advanced studies are potentially required to evaluate the protective effect of dietary WPCP for *O. niloticus* under high fish stocking density, and different stress conditions, and determine its role as a reproductive enhancer agent for different fish species in the adult and/or brood stock stages. This may be adjusted to improve fish health and reproductive performance, and may hence, increase fish productivity and profitability in fish farms and hatcheries.

**REFERENCES**


Whey protein as a growth promoter and an immunostimulant agent for *O. niloticus*


