Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 25(4): 1017 – 1033 (2021)
www.ejabf.journals.ekb.eg

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

Ahmed I. Mehrim[1*], Mohamed M. Refaey[1] and Osama A. Zenhom[2]
1 Animal Production Department, Faculty of Agriculture, Mansoura University, Egypt.
2 Central Laboratory for Aquaculture Research, Abbasa, Abo-Hammad, Egypt.
[*]Corresponding Author: amehrim2002@yahoo.com

ARTICLE INFO
Article History:
Received: July 8, 2021
Accepted: July 27, 2021
Online: Aug. 30, 2021

Keywords:
Aquaculture,
Nile tilapia,
Whey protein,
Feed additives,
Growth,
Immune.

ABSTRACT

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.

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 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 improvments 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

Ingredient	**%**
Fish meal (72% CP)	8
Corn gluten (60% CP)	15
Soybean meal (44% CP)	28
Wheat bran	20
Yellow corn	20
Molasses	3
Corn Oil	5
Vitamins and minerals premix[1]	1
Nutrient composition	
Dry matter (DM, %)	93.53
Crude protein (CP, %)	30.91
Ether extract (EE, %)	7.23
Ash (%)	5.64
Total carbohydrates (%)	56.22
Gross energy (GE, MJ 100 g^{-1} DM)[2]	2004.3

[1] Each 3 Kg premix contains: Vit. A, 12000,000 IU; Vit. D_3, 3000,000 IU; Vit. E, 10,000 mg; Vit. K_3, 3000 mg; Vit. B_1 200 mg; Vit. B_2, 5000 mg; Vit. B_6, 3000 mg; Vit. B_{12}, 15 mg; Biotin, 50 mg; Folic acid, 1000 mg; Nicotinic acid, 35000 mg; Pantothenic acid, 10,000 mg; Mn, 80g; Cu, 8.8g; Zn, 70 g; Fe, 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) = $\mathbf{FW\ (g) - IW\ (g)}$.

Average daily gain (ADG, g $fish^{-1}$ day^{-1}) = $\frac{\mathbf{TWG}}{\mathbf{T}}$.

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

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

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

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

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) = $\frac{\text{TWG}}{\text{protein intake}}$.

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 compostion 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 × 10^6 mm^{-3}), blood platelets (× 10^3 mm^{-3}), total white blood cells (WBCs × 10^3 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 (T_0-T_{20}), 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 T_{20} significantly increased all the growth performance parameters among all the experimental treatments ($P \leq 0.05$). While the same treatment (T_{20}) led to an insignificant increase of SR and K-factor among to the other treatments ($P \geq 0.05$).

Table 2: Effect of different levels of dietary whey protein concentrate powder on growth performance parameters of *Oreochromis niloticus*

Traits	Treatments			
	T_0	T_5	T_{10}	T_{20}
Final weight (g)	39.64±0.53^b	43.80±1.94^b	43.53±1.55^b	50.74±0.42^a
Total weight gain (g)	22.89±0.53^b	27.05±1.94^b	26.78±1.55^b	33.99±0.42^a
Average daily gain (g $fish^{-1}$ day^{-1})	0.38±0.01^b	0.45±0.03^b	0.45±0.03^b	0.57±0.01^a
Relative growth rate (%)	136.7±3.19^b	161.4±11.60^b	159.8±9.27^b	202.9±2.53^a
Specific growth rate (% day^{-1})	1.44±0.02^b	1.60±0.08^b	1.59±0.06^b	1.85±0.01^a
Survival rate (%)	90.47±4.76	90.38±4.81	95.24±4.76	100.0±0.00
Condition factor (K, %)	1.74±0.05	1.78±0.04	1.80±0.06	1.77±0.03

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 (T_0) are showed in Table (3). Fish fed supplemented diet with different levels of WPCP, especially at the high level (T_{20}) 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 ($P \leq 0.05$).

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

Traits	Treatments			
	T_0	T_5	T_{10}	T_{20}
Feed intake (g)	66.87±3.31	67.50±1.40	66.09±3.79	72.04±4.61
Feed conversion ratio	$2.92±0.16^a$	$2.51±0.14^{ab}$	$2.49±0.22^{ab}$	$2.12±0.13^b$
Protein efficiency ratio	$1.11±0.06^b$	$1.29±0.07^{ab}$	$1.32±0.12^{ab}$	$1.54±0.10^a$
Protein productive value (%)	$17.44±2.14^b$	$21.77±1.43^{ab}$	$20.05±2.39^{ab}$	$28.21±1.82^a$
Energy utilization (%)	$11.15±0.46^b$	$13.82±0.73^{ab}$	$11.35±1.27^b$	$16.24±1.27^a$

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

3. The chemical composition of the fish

Fish fed a high level of WPCP (T_{20}) significantly increased ($P \leq 0.05$) both dry matter and crude protein compared to T_0 (Table 4). However, fish in T_5 displayed significantly increased crude fat and energy content among all treatments. Meanwhile, the control treatment (T_0) showed significantly increased ash content among all treatments ($P \leq 0.05$).

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

Treatments	Dry matter (%)	On dry matter basis			
		Ash (%)	Fat (%)	Protein (%)	Energy content (MJ 100 g^{-1} DM)
T_0	$22.97±1.06^b$	$18.46±0.34^a$	$18.68±0.26^b$	$62.67±0.08^b$	$2227±12.00^c$
T_5	$24.65±0.57^{ab}$	$15.18±0.09^c$	$22.81±0.40^a$	$62.00±0.36^b$	$2367±7.68^a$
T_{10}	$21.92±0.40^c$	$16.33±0.40^b$	$19.71±0.62^b$	$63.95±0.45^a$	$2291±17.57^b$
T_{20}	$25.84±0.58^a$	$16.27±0.29^b$	$18.84±0.13^b$	$64.89±0.36^a$	$2278±6.33^b$

Different small letters in the same column indicate significant differences among treatments ($P \leq 0.05$). 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 (T_{10} and T_{20}) significantly increased both RBCs and Hb compared to those in T_5 or T_0 (Fig. 1), where $P \leq 0.05$. However, T_{20} showed a significant increase of PCV and blood platelets among all treatments ($P \leq 0.05$).

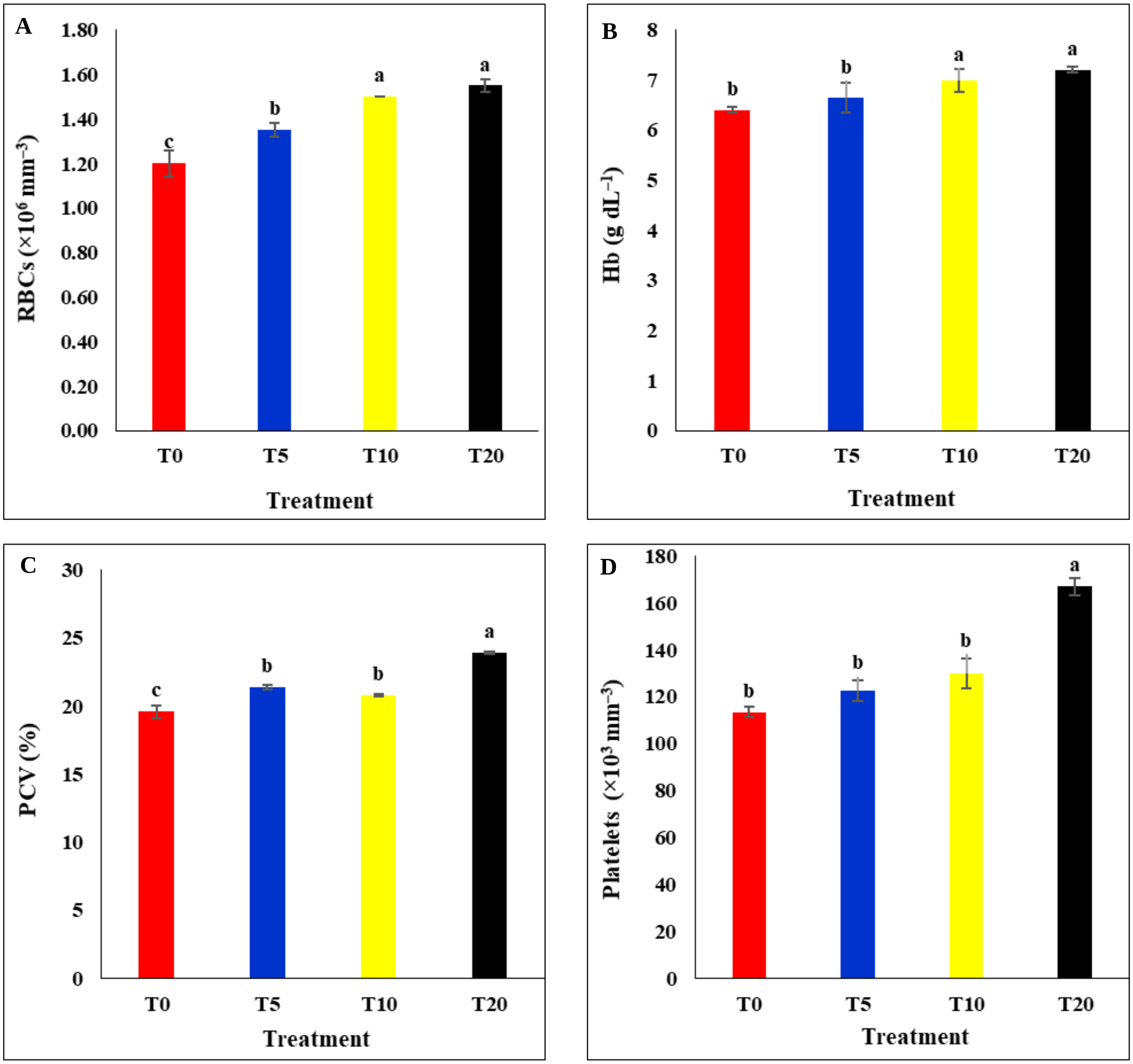
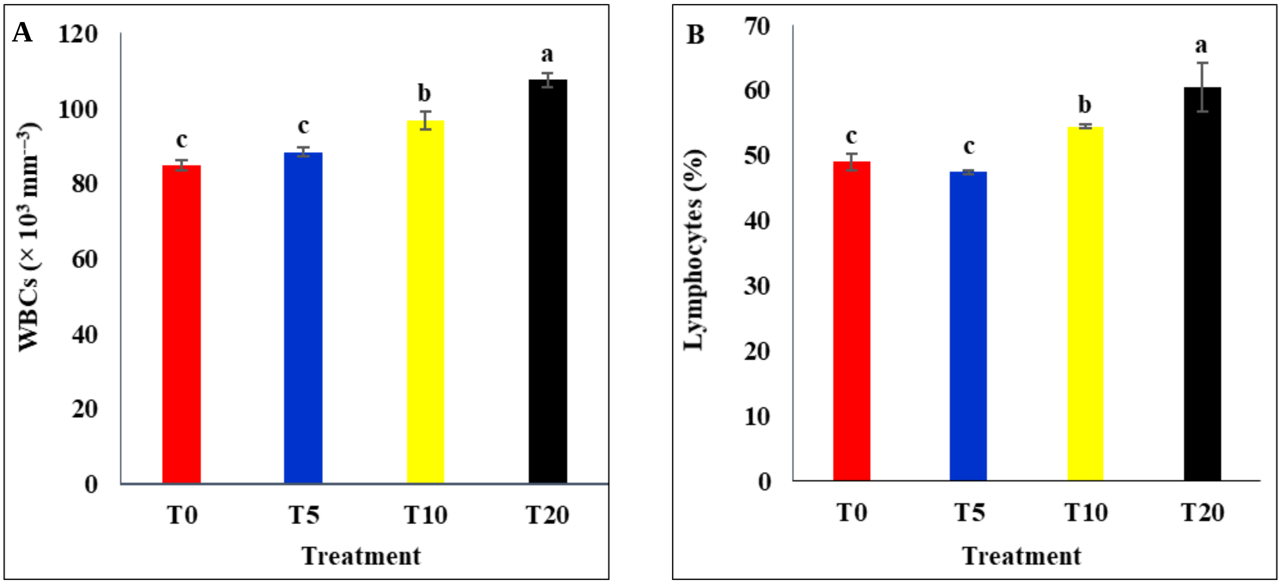


Fig. 1: Effect of different levels of dietary whey protein concentrate powder on hematological parameters (A) Red blood cells (RBCs), (B) Hemoglobin (Hb), (C) Packed cell volume (PCV), and (D) Platelets of *Oreochromis niloticus*. Vertical bars indicate the standard error. Small letters indicate significant differences among treatments ($P \leq 0.05$).

5. Immune indicators measurements

Immune responses parameters (as WBCs, lymphocytes, and IgM) significantly increased with the increasing levels of WPCP (Fig. 2), where $P \leq 0.05$. The T_{20} recorded the highest values of immune responses parameters compared to other treatments.



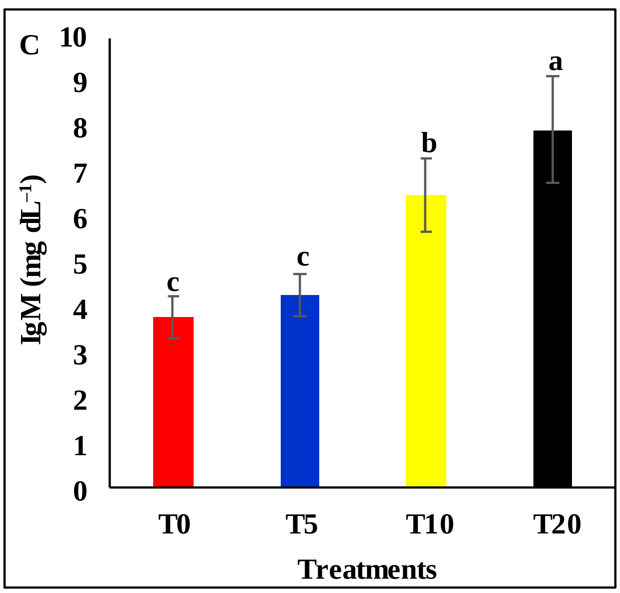


Fig. 2: Effect of different levels of dietary whey protein concentrate powder on immune indicators parameters (A) White blood cells (WBCs), (B) Lymphocytes, and (C) Serum immunoglobulin M (IgM) of *Oreochromis niloticus*. Vertical bars indicate the standard error. Small letters indicate significant differences among treatments ($P \leq 0.05$).

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 T_{10} and T_{20}, 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 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 (T_{20}) 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 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 T_{10} and T_{20}, 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 (**Rutherfurd-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 (T_{20}) 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 worthnoting 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

Abdelhamid, M. A.; Mehrim, A.I.; El-Barbary, M.I. and El-Sharawy, M.A. (2013). Effect of some commercial feed additives on the structure of gonads and microbiology of Nile tilapia (*Oreochromis niloticus*) fish. Egypt. J. Aquat. Biol. & Fish., 17 (2): 47 – 62.

Abdel-Tawwab, M. and Abbass, F. E. (2016). Dry whey meal as a protein source in practical diets for Nile tilapia, *Oreochromis niloticus* fingerlings. J. Appl. Aquac., 28 (4): 276-284.

Afkhami, M.; Kermanshahi, H. and Heravi, R. M. (2020). Evaluation of whey protein sources on performance, liver antioxidants and immune responses of broiler chickens challenged with ethanol. J. Anim. Physiol. Anim. Nutr., 00:1–11.

Akhavan, T.; Luhovyy, B.L.; Brown, P.H.; Cho, C.E. and Anderson, G.H. (2010). Effect of premeal consumption of whey protein and its hydrolysate on food intake and postmeal glycemia and insulin responses in young adults. Am. J. Clin. Nutr., 91: 966-975.

Alemayehu, T. A.; Geremew, A. and Getahun, A. (2018). The role of functional feed additives in tilapia nutrition. Fish. Aqua. J., 9:(2): 1-6.

Alloui, M. N. and Szczurek, W. (2017). Effects of different dietary levels of whey lactose as a prebiotic disaccharide on the productive performances and selected indices of the caecal micro-environment in broiler chickens. Ann. Anim. Sci., 17: 1107–1122.

Amer, S. A.; Osman, A.; Al-Gabri, N.A.; Elsayed, S.A.M.; Abd El-Rahman, G.I.; Elabbasy, M. T.; Ahmed, S.A.A. and Ibrahim, R.E. (2019). The effect of dietary replacement of fish meal with whey protein concentrate on the growth performance, fish health, and immune status of Nile tilapia fingerlings, *Oreochromis niloticus*. Animals, 9: 1003.

Anthony, J.C.; Anthony, T.G.; Kimball, S.R. and Jefferson, L.S. (2001). Signaling pathways involved in translational control of protein synthesis in skeletal muscle by leucine. J. Nutr., 131:856S-860S.

AOAC (2004). Official method of Analysis of the Association of official Analytical chemists. AOAC International, Washington DC.

Asimi, O.A. and Sahu, N.P. (2013). Herbs/spices as feed additive in aquaculture. J. Pure Appl. Sci. Technol., 2(8):284-292.

Athira, S.; Mann, B.; Sharma, R. and Kumar, R. (2013). Ameliorative potential of whey protein hydrolysate against paracetamol-induced oxidative stress. J. Dairy Sci., 96: 1431-1437.

Ayhan, V.; Diler, I.; Arabacı, M. and Sevgili, H. (2018). Enzyme supplementation to soybean-based diet in gilthead sea bream (*Sparus aurata*): Effects on growth parameters and nitrogen and phosphorus excretion. Kafkas Univ. Vet. Fak., (14): 161–168.

Barrows, F. T. and Frost, J. B. (2014). Evaluation of the nutritional quality of co-products from the nut industry, algae and an invertebrate meal for rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 434:315–324.

Bell, S. J. (2000). Whey protein concentrates with and without immunoglobulins: A Review. J. Med. Food, 3 (1): 1-13.

Blome, R.M.; Drackley, J.K.; McKeith, F.K.; Hutjens, M.F. and McCoy, G.C. (2003). Growth, nutrient utilization, and body composition of dairy calves fed milk replacers containing different amounts of protein. J. Anim. Sci., 81: 1641-1655.

Boyd, C. E. and Tucker, C. S. (2014). Handbook for Aquaculture Water Quality. Craft master Printers, Inc., Auburn, Alabama, 439pp.

Coker, R.H.; Miller, S.; Schutzler, S.; Deutz, N. and Wolfe, R.R. (2012). Whey protein and essential amino acids promote the reduction of adipose tissue and increased muscle protein synthesis during caloric restriction-induced weight loss in elderly, obese individuals. Nutr. J., 11: 105.

Dacie, J. V. and Lewis, S. M. (1995). Practical Haematology. Eighth ed., Churchill Livingstone, Edinburgh.

Dada, A.A. (2015). Improvement of tilapia (*Oreochromis niloticus* Linnaeus, 1758) growth performance fed three commercial feed additives in diets. J. Aquac. Res. Dev., 6: 325-327.

Eason, R. R.; Velarde, M. C.; Chatman, L.; Till, S. R.; Geng, Y.; Ferguson, M. and Simmen, R.C.M. (2004). Dietary exposure to whey proteins alters rat mammary gland proliferation, apoptosis, and gene expression during postnatal development. Nutr. J., 134 (12): 3370–3377.

Ebru, Y. and Cengiz, K. (2016). Feed additives in aquafeeds. Anim. Sci. J., 66: 155-160.

El-Sayed, A. F. M. (2006). Tilapia culture in salt water: environmental requirements, nutritional implications and economic potentials. Avances en Nutricion Acuicola, 95-106.

El-Shewy, A.A. (2016). Whey as a feed ingredient for lactating cattle. Sci. Int., 4: 80-85.

Expósito, I.L. and Recio, I. (2006). Antibacterial activity of peptides and folding variants from milk proteins. Int. Dairy J., 16:1294–1305.

FAO (2020). The State of World Fisheries and Aquaculture 2020. Sustainability in action. Rome, 224pp.

GAFRD (2019). Fisheries statistics yearbook 2019. 29th Edition. General Authority for Fish Resources Development, Cairo, 104pp.

Galeotti, M.; Romano, N.; Volpatti, D.; Bulfon, C.; Brunetti, A.; Tiscar, P.G.; Mosca, F.; Bertoni, F.; Marchetti, M.G. and Abelli, L. (2013). Innovative vaccination protocol against vibriosis in *Dicentrarchus labrax* (L.) juveniles: Improvement of immune parameters and protection to challenge. Vaccine, 31:1224–1230.

Gaudel, C.; Nongonierma, A.B.; Maher, S.; Flynn, S. and Krause, M. (2013). A whey protein hydrolysate promotes insulinotropic activity in a clonal pancreatic Î²-cell line and enhances glycemic function in ob/ob mice. J. Nutr., 143: 1109-1114.

Gauthier, S.F.; Pouliot, Y. and Saint-Sauveur, D. (2006). Immunomodulatory peptides obtained by the enzymatic hydrlysis of whey proteins. Int. Dairy J., 16: 1315–1323.

Grinstead, G.S.; Goodband, R.D.; Dritz, S.S.; Tokach, M.D.; Nelssen, J.L.; Woodworth, J.C. and Molitor, M. (2000). Effects of a whey protein product and spray-dried animal plasma on growth performance of weanling pigs. J. Anim. Sci., 78 (3): 647–657.

Groleau, P.E.; Morin, P.; Gauthier, S.F. and Pouliot, Y. (2003). Effect of physicochemical conditions on peptide-peptide interactions in a tryptic hydrolysate of β-lactoglobulin and identification of aggregating peptides. J. Agric. Food Chem., 51: 4370–4375.

Hossain, M.A.; Pandey, A. and Satoh, S. (2007). Effects of organic acids on growth and phosphorus utilization in red sea bream *Pagrus major*. Fish. Sci., 73(6):1309-1317.

Jazi, V.; Farahi, M.; Khajali, F.; Abousaad, S.; Ferket, P. and Assadi Soumeh, E. (2020). Effect of dietary supplementation of whey powder and *Bacillus subtilis* on growth performance, gut and hepatic function, and muscle antioxidant capacity of Japanese quail. J. Anim. Physiol. Anim. Nutr., 00:1–12.

Jensen, G.S.; Patel, D. and Benson, K.F. (2012). A novel extract from bovine colostrum whey supports innate immune functions. II. Rapid changes in cellular immune function in humans. Prev. Med., 54 Suppl: 124-129.

Kermanshahi, H. and Rostami, H. (2006). Influence of supplemental dried whey on broiler performance and cecal flora. Int. J. Poult. Sci., 5: 538–543.

Khajepour, F. and Hosseini, S.A. (2012). Citric acid improves growth performance and phosphorus digestibility in Beluga (*Huso huso*) fed diets where soybean meal partly replaced fishmeal. Anim. Feed Sci. Tech., 171 (1):68-73.

Khaled, M. (2015). Effect of organic acid salt supplementation on growth performance and feed utilization in practical diets of hybrid tilapia (♀ *O. niloticus* × ♂ *O. aureus*) fingerlings. Egypt. J. Anim. Prod., 52:81-88.

Khalil, F.F.; Farrag, F.H.; Mehrim, A.I. and Refaey, M.M. (2014). Pawpaw (*Carica papaya*) seeds powder in Nile tilapia (*Oreochromis niloticus*) diets: 2. Liver enzymes, sexual hormones and histological structure of the gonads. Egypt. J. Aquat. Biol. & Fish., 18 (1): 97-113.

Khalil, F.F.; Mehrim, A.I. and Refaey, M.M. (2015). Impact of dietary rocket (*Eruca sativa*) leaves or seeds on growth performance, feed utilization, biochemical and physiological responses of *Oreochromis niloticus*, fingerlings. Asian J. Anim. Sci., 9 (4): 134-147.

Knowles, G. and Gill, H. (2004). Immunomodulation by dairy ingredients: Potential for improving health. In Handbook of Functional Dairy Products; CRC Press: Boca Raton, Florida, pp. 125–153.

Lovell, R. T. (2001). Nutrition and Feeding of Fish. Third ed., Haworth Press, New York, 267pp.

Low, P.; Rutherfurd, K.; Cross, M. and Gill, H. (2001). Enhancement of mucosal antibody responses by dietary whey protein concentrate. Food Agric. Immunol., 13: 255–264.

Low, P.; Rutherfurd, K.; Gill, H. and Cross, M. (2003). Effect of dietary whey protein concentrate on primary and secondary antibody responses in immunized BALB/c mice. Int. Immunopharmacol., 3: 393–401.

Mazurkiewicz, J.; Przybył, A. and Golski, J. (2008). Usability of Fermacto prebiotic in feeds for common carp (*Cyprinus carpio* L.) fry. Nauka Przyroda Technologie., 2(3):15.

Mehrim, A.I. (2009). Effect of dietary supplementation of Biogen® (commercial probiotic) on mono-sex Nile tilapia, *Oreochromis niloticus* under different stocking densities. J. Fish. Aquat. Sci., 4 (6): 261-273.

Mehrim, A.I. (2012). Effect of dietary chromium picolinate supplementation on growth performance, carcass composition and organs indices of Nile tilapia (*Oreochromis niloticus* L.) fingerlings**.** J. Fish. Aquat. Sci., 7 (3): 224-232.

Mehrim, A.I. (2014). Physiological, biochemical and histometric responses of Nile tilapia (*Oreochromis niloticus* L.) by dietary organic chromium (chromium picolinate) supplementation. J. Adv. Res., 5: 303-310.

Mehrim, A.I.; Abdelhamid, M.A.; Ibrahim, S.M. and Abd El-Wahab, A.I. (2013). Assessment of a new local prebiotic impacts on the reproductive efficiency of African catfish (*Clarias gariepinus* Burchell, 1822) brood stock. Journal of the Arabian Aquaculture Society, 8 (1): 105 – 120.

Mehrim, A.I.; Khalil, F. F. and Hassan, M. E. (2015). Hydroyeast Aquaculture® as a reproductive enhancer agent for the adult Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758). Fish Physiol. Biochem., 41:371–381.

Mehrim, A.I.; Khalil, F.F. and Hassan M.E. (2019a). Sexual maturity signs and histological alterations of adult *Oreochromis niloticus* (Linnaeus, 1758) fed probiotic. Int. J. Anat. Appl. Physiol., 5(1):103-110.

Mehrim, A.I.; Khalil, F.F. and Refaey, M.M. (2014). Evaluation of dietary addition of garlic (*Allium sativum* L.) lobes on growth performance, feed utilization, and physiological responses of *Oreochromis niloticus*, fingerlings. Abbassa International Journal for Aquaculture, 7 (2): 342 – 361.

Mehrim, A.I.; Khalil, F.F.; Farrag, F.H. and Refaey, M.M. (2019b). 17α-methyltestosterone and some medicine plants as reproductive controller agents of *Oreochromis niloticus*. J. Bio. Sci., 19 (6): 407 - 417.

Mehrim, A.I.; Khalil, F.F.; Refaey, M.M. and Abdalqadir, A.M. (2017). Impacts of Pro-Grow® on growth performance, physiological, immune responses and economic efficiency of adult *Oreochromis niloticus* (Linnaeus, 1758) under stocking density stress. Sci. Int., 5 (3): 113-126.

Morifuji, M.; Kensuke, S. and Sugiura, K. (2005). Dietary whey protein modulates liver glycogen level and glycoregulatory enzyme activities in exercise-trained rats. Exp. Biol. Med., 230(1):23-30.

Morken, T.; Kraugerud, O.F.; Barrows, F.T.; Sørensen, M.; Storebakken, T. and Øverland, M. (2011). Sodium diformate and extrusion temperature affect nutrient digestibility and physical quality of diets with fishmeal and barley protein concentrate for rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 317:138-145.

NRC (1994). Nutrient requirements of poultry. Ninth ed., National Academy Press, Washington DC, 176pp.

Pihlanto, A. (2006). Antioxidative peptides derived from milk proteins. Int. Dairy J., 16: 1306–1314.

Pineda-Quiroga, C.; Camarinha-Silva, A.; Borda-Molina, D.; Atxaerandio, R.; Ruiz, R. and García-Rodríguez, A. (2018). Feeding broilers with dry whey powder and whey protein concentrate affected productive performance, ileal digestibility of nutrients and cecal microbiota community. Animal, 12: 692–700.

Rutherfurd-Markwick, K.J.; Johnson, D.; Cross, M.L. and Gill, H.S. (2005). Modified milk powder supplemented with immunostimulating whey protein concentrate (IMUCARE) enhances immune function in mice. Nutr. Res., 25: 197–208.

Saito, T. (2008). Antihypertensive peptides derived from bovine casein and whey proteins. Adv. Exp. Med. Biol., 606: 295-317.

SAS (2006). SAS 9.1.3 procedure user's guide. SAS Institute Inc., Cary, North Carolina.

Smilowitz, J.T.; Dillard, C.J. and German, J.B. (2005). Milk beyond essential nutrients: the metabolic food. Aust. J. Dairy Technol., 60: 77-83.

Stoskopf, M. K. (1993). Fish Medicine. Saunders, W. B. Company, Philadelphia.

Sukkar, S. G. and Bounous, U. G. (2004). The role of whey protein in antioxidant defense. Rivista Italiana di Nutrizione Parenterale ed Enterale, 22(4): 193-200.

Tavares, T.G. and Malcata, F.X. (2013). Whey proteins as source of bioactive peptides against hypertension. Bioact. Food Pept. Health Dis., 2013: 75–114.

Tsakali, E.; Petrotos, K.; D'Allessandro, A. and Goulas, P. (2010). A review on whey composition and the methods used for its utilization for food and pharmaceutical products. In Proceedings of the 6th International Conference on Simulation and Modelling in the Food and Bio-industry: FOODSIM, Bragança, Portugal, 24–26 June 2010; pp. 195–201.

Udenigwe, C.C. and Aluko, R.E. (2012). Food protein-derived bioactive peptides: Production, processing, and potential health benefits. J. Food Sci., 77: R11–R24.

Walrand, S.; Zangarelli, A.; Guillet, C.; Salles, J. and Soulier, K. (2011). Effect of fast dietary proteins on muscle protein synthesis rate and muscle strength in ad libitum-fed and energy-restricted old rats. Br. J. Nutr., 106: 1683-1690.

Walzem, R.L.; Dillard, C.J. and German, J.B. (2002). Whey components: millennia of evolution create functionalities for mammalian nutrition: what we know and what we may be overlooking. Crit. Rev. Food Sci. Nutr., 42:353-375.

West, N.P.; Pyne, D.B.; Cripps, A.W.; Christophersen, C.T.; Conlon, M.A. and Fricker, P.A. (2012). Gut Balance, a synbiotic supplement, increases fecal *Lactobacillus paracasei* but has little effect on immunity in healthy physically active individuals. Gut Microbes., 3: 221-227.

Wolfe, R. R. (2000). Protein supplements and exercises. Am. J. Clin. Nutr., 72: 551-557.

Wong, K.; Middleton, N.; Montgomery, M.; Dey, M. and Carr, R. (1998). Immunostimulation of murine spleen cells by materials associated with bovine milk protein fractions. J. Dairy Sci., 81: 1825–1832.

Xu, R.; Liu, N.; Xu, X. and Kong, B. (2011). Antioxidative effects of whey protein on peroxide-induced cytotoxicity. J. Dairy Sci., 94: 3739-3746.

Yoshizawa, F. (2004). Regulation of protein synthesis by branched-chain amino acids *in vivo*. Biochem. Biophys. Res. Commun., 313: 417–422.

Yousefi, S.; Hoseinifar, S.H.; Paknejad, H. and Hajimoradloo, A. (2018). The effects of dietary supplement of galactooligosaccharide on innate immunity, immune related genes expression and growth performance in zebrafish (*Danio rerio*). Fish Shellfish Immun., 73:192-196.

Zemel, M.B. (2004). Role of calcium and dairy products in energy partitioning and weight management. Am. J. Clin. Nutr., 79 (Suppl.): 907S-912S.

Zheng, C.C.; Wu, J.W.; Jin, Z.H.; Ye, Z.; Yang, S.; Sun, Y. and Fei, H. (2019). Exogenous enzymes as functional additives in finfish aquaculture. Aquacult. Nutr., 00:1–12.