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Biochemical and Nutritional Studies on the Nile Tilapia Fed Orange Peel Additive

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ABSTRACT

The present study recorded a good trial for using orange peel as a natural additive to enhance the growth, the physiological, biochemical, and immunity measurements of the mono-sex Nile tilapia. Orange peel additive, 2% dried powder, 2% oil extraction, and 2% mixture of them (1:1); diet 1, diet 2, and diet 3, respectively, were used for feeding three test groups of fish besides to the control (0% additive). The best growth performance, feed utilization, and blood measurements were recorded for the three treated groups especially for those fed diet 3, which contained the mixture when compared to the control. Orange peel additive increased ($p<0.05$) body protein and plasma total protein, while blood enzymes activity, lipid content, and cholesterol were decreased ($p<0.05$). Immunological blood parameters (IgG & IgM) were enhanced for fish fed the additive. The present results determined that the immune response system was improved for fish fed the forms of orange peel.

INTRODUCTION

The tilapias are considered the second largest farmed finfish class after carps in the world **(FAO, 2017)**. The last decades have witnessed an outstanding global extension in *Oreochromis niloticus* culture almost throughout 100 countries in Asia and Africa **(Gu *et al.*, 2017)**. Notably, global tilapia production has increased from 0.5 to 5.7 million metric tons during 1990 till 2015, with an estimated annual average improving rate of 13.5% **(FAO, 2017)**.

The use of economic unconventional diets and inexpensive feed sources are considered major targets of fish nutritionists. In Egyptas well as in many Mediterranean countries, a huge quantity of the citrus peels is not processed, and some efforts were made for using these by-products as animal feed **(Chedea *et al.*, 2010; Farhat *et al.*, 2011)**. Citrus by-products are considered easy, available and low-cost nutritional dietary supplements, which can provide an efficient, cheap, and environment friendly platform for the production of new nutritional resources **(Rafiq *et al.*, 2016)**. Orange peel is a rich source of phenolic compounds and fibre due to the large amount of peel produced. Citrus

(*citrus spp*) belonging to the family Rutaceae, is one of the most plentiful fruit crops with world production that reaches almost 115 million tons per year, among which sweet oranges, lemon, tangerines or mandarin, and grape fruits are considered **(Olabinjo *et al.*, 2017)**.

Sweet orange, *Citrus sinensis*, is one of the most common fruits in the world. It is used for juiceand jam productionleavinggreat amounts of peels, seeds, and pulps, addingly it represents 50% of the raw fruit **(Li *et al.*, 2006; Anwar *et al.*, 2008)**. Orange and its wastes are important sources of phytochemicals **(Genovese *et al.*, 2014)** that can protect human, animal, and may also be healthy for fish providing affluent supply of vitamin C, folic acid, potassium, and pectin. Orange peel contains higher concentration of ascorbic acid and has much active materials compared to its juice and pulp **(Hakim & Harris, 2001; Anagnostopoulou *et al.*, 2006; Guimaraes *et al.*, 2009)**.

Active materials of orange peel i.e. alkaloids, flavonoids, phenols, terpenes, resins, limonene, phytate, tannins oxalate pigments, steroids, and essential oils which improve growth and other biological activities (antistress, appetite, immunity, and antimicrobial properties) **(Oluremi *et al.*, 2007; Al-Saadi *et al.*, 2009; Citarasu, 2010)**. In addition, orange peels contain some important elements, such as phosphorus, chromium, zinc, manganese, copper and iron **(Al-Saadi *et al.*, 2009)**. Orange oil extraction constitutes almost 13 % of peels and contains around 90% volatile and 10% non-volatile components **(Smith *et al.*, 2001)**.

Citrus oils is a particularly interesting field for applications within the food **(Crowel, 1999)**. Orange peel oil has a highly D-limonene content (84-96%) which has positive effect on fish growth **(Gültepe *et al.*, 2020)**. The main objectives of this research was to use orange peel dried powder and extract essential oil from the peel (*citrus sinensis*) to be used as additive for feeding the tilapia.

MATERIALS AND METHODS

Experimental design

An experiment was conducted for 14 weeks to undergo a fish treatment using orange peel as an additive for the Nile tilapia monosex feed, with an initial body weight of 17.0 ± 0.5 g.

A concrete pond of 40 m^3(4×10×1.5m), was divided into 8 equal parts by using plastic nets,; each of which contained100 fish. The 4 duplicate parts represented 4 dietary treatments as follows: diet 1 (2% orange peel powder), diet 2 (2% orange peel oil), diet 3 (2% mixture of powder and oil), and the control (0% additives). The test diet formula and chemical compositionare shown in Table (1 & 2).

For acclimatization purpose, fish samples were held under optimal conditions for a 2 weeks- period before starting the trial. Fish were fed twice a day at 9:30 am and 13:30

pm. Fish weight was calculated biweekly to adjust daily feeding rate (3%). Furthermore, water was partially changed twice a week.

Table 1. The formula of experimental diet

Ingredients (%)	Experimental Diets			
	Control	Diet 1 Powder	Diet 2 Oil	Diet 3 Mixture
Fish Meal	15.0	15.0	15.0	15.0
Soybean Meal	30.0	30.0	30.0	30.0
Corn Gluten Meal	3.0	3.0	3.0	3.0
Yellow Corn	23.0	21.0	21.0	21.0
Wheat Bran	23.0	23.0	23.0	23.0
Sun Flower Oil	2.0	2.0	2.0	2.0
Nacl	2.0	2.0	2.0	2.0
Premix*	2.0	2.0	2.0	2.0
Dried Orange Peel	-	2.0	-	-
Orange Oil	-	-	2.0	-
Mixture (1:1)	-	-	-	2.0

*One kg premix contained:

Vitamins: 48×10^5 I.U (A), 6×10^2 mg (B_6), 20 mg (biotin), 8×10^5 I.U. (D_3), 144 mg (E), 400 mg (B_1), 1600 mg (B_2), 4×10^3 mg (pantothenic acid), 4 mg (B_{12}), 4×10^2 mg (niacin), 2×10^5 mg (choline chloride), and 400 mg (folic acid).

Minerals: 12×10^3 mg iron, 16×10^3 mg manganese, 12×10^2 mg copper, 120 mg iodine, 80 mg cobalt, 40 mg selenium, and 16×10^3 mg zinc.

Water quality parameters

During the experimental period, water quality parameters, such as water temperature, dissolved oxygen, and pH were measured daily. Nitrite, nitrate and unionized ammonia were weekly calculated according to the methodof **Zhang *et al.* (2018)**

Table 2. Chemical composition of expermintal diets

Chemical Composition (%)	Experemintal Diets			
	Control	Diet 1	Diet 2	Diet 3
Dry Matter	89.65	88.26	89.91	89.09
Crude Protein (CP)	28.55	28.56	28.40	28.48
Ether Extract (EE)	5.16	5.11	7.07	6.09
Crude Fibre (CF)	4.34	5.16	4.34	4.75
Nitrogen Free Extract (NFE)*	41.26	40.74	39.80	40.27
Ash	6.19	6.23	6.17	6.20
GE (MJ/kg)**	16.84	16.87	17.32	17.09

***NFE** = 100-(CP% + EE% + CF% + Ash%).

****GE** =Gross Energy calculated as 5.65, 9.65, 4.2,and 4.2 Kcal/g of protein, lipid, fibre, and carbohydrates, respectively **(NRC, 2011).**

Feed additive preparation

Orange peels were obtained from Hero food industries (Vitrac, Tersa, Qalubia governorate), and dried at 50ºC for 48 h. Orange peel powder was prepared using a mechanical laboratory grinder and passed through sieve mesh 24 µm, then packaged in polyethylene bags and stored at 4±1°C until required for use as additive in fish diet. Chemical composition and mineral content of the powderare shown in Tables (3 & 4).

Oil was obtained from the local market produced by El-Captain company (Cap Pharm for extracting oils, herbs, and Cosmotics, El Obour city, Cairo, Egypt. (www.elcaptain-eg.com).

Determination of Vitamin (C) in orange peel

The powder of orange peel (1g) was extracted in 4 % oxalic acid and the solution brought to 100 ml (V1 ml) and centrifuged at 4032 xg for 10 min. Then, 5 ml of the supernatant was mixed with10 ml of 4 % oxalic acid and the solution was titrated against the dye (V2 ml). Ascorbic acid mg/100g = 0.5mg/V1 ml ×V2/5 ml ×100 ml/sample weight (1g) ×100.

Ascorbic acid mg/100g = 0.5mg/V1 ml ×V2/5 ml ×100 ml/sample weight (1g) ×100. (**Nwanna *et al*., 2011)**.

Table 3. Chemical composition of dried orange peel (Dry matter)

Chemical Parameters	**Orange Peel**
Moisture %	83.5
Crude Fibre %	40.75
Crude Protein%	7.94
Ash %	3.01
Fat %	0.95
NFE %	47.35
Vitamin C (g/kg)	227.8

Apparent protein digestibility (APD) was measured by using the method of **NRC (1993).** The wastes (uneaten diet and faeces) were collected by siphoning once daily throughout the last 15 days of the experimental period. Any feed or faeces was carefully collected before first feeding. After about 30 min of feeding, uneaten feed was collected. Faeces collected separately after 2 hours, filtered then dried at 60ºC and kept for subsequent chemical composition.

Physiological parameters

a. Body indices

Livers and viscera of experimental fishwere taken at the end of the feeding trail and were weighed to calculate hepato-somatic index (HSI) and gut index (GSI) as follows:

HSI = (liver weight/fish total weight) x100

GSI = (gut weight/fish total weight) x100

b. Blood parameters

At the end of experiment, blood samples were collected from the fish caudal vein using heparinized syringes. Blood was centrifuged at 1008 x*g* for 15 min. Sample were subjected to the determination of plasma protein (PTP) following the method of **Armstrong and car (1964)**. Serum cholesterol was determined according to the method described by **Stein (1986).** Glucose concentrates were measured according to the method of **Trainder (1969).** Immunoglobulins (IgM and IgG) were determined according to method of **Feinstein *et al.* (1985)**.

Table 4. Minerals content of orange peel powder

Element	Concentration (ppm)
Potassium (K)	157.1
Iron (Fe)	123.7
Manganese (Ma)	87.2
Calcium (Ca)	41.6
Sodium (Na)	22.8
Zinc (Zn)	13.4
Magnesium (Mg)	4.43
Nickel (Ni)	1.48
Copper (Cu)	1.11
Chromium (Cr)	1.23
Lead (Pb)	0.24
Phosphorus (P)	0.22
Cadmium (Cd)	0.10

Fish growth performance and feed utilization parameters were calculated according to **Cho and Kaushik (1985)** as the following:

Body weight gain (**BWG**, g/fish) = [final body weight (g) – initial body weight (g)]

Daily weight gain, (**DWG**, g/fish/day) = [BWG (g) / Experimental period (days)]

Specific growth rate (**SGR**, %g/day) = [Ln final weight – Ln initial weight] / Experimental period (day)×100

Feed conversion ratio (**FCR**) = feed intake (g) / body weight gain (g)

Protein efficiency ratio (**PER**) = gain in weight (g) / protein intake in feed (g)

Protein productive value (**PPV**, %) =100 [protein gain in fish (g) / feed protein intake (g)]

Fish Survival rate % = 100[Initial number of fish stocked-Mortality] / Initial number of fish stocked.

Chemical analysis

Chemical analysis of additives, experimental diets, and fish body were determined dry matter base (DM %).**)**. Crude protein (CP %), ether extract (EE %), crude fibre (CF

%), and ash (%), in addition to to the concentrations of the minerals in the orange peel powder were measured according to the **AOAC method (2012**).

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using general linear models (GLM) procedure; the software used was SPSS (Version 16.0) **(SPSS, 1997)**. Duncan's multiple range tests **(Duncan, 1955)** was used to compare between means of the control and those of the treated groups.

The model of analysis was as follows:

$\mathbf{Y_{ij} = \mu + T_i + E_{ij}}$

μ = the overall mean,

T_i = the effect of treatment,

and, E_{ij} = the random error.

RESULTS AND DISCUSSION

Water quality

The mean values of water quality (±SD)are recorded in Table (5). All water quality parameters were within the acceptable ranges for rearing the tilapia **(Makori *et al.*, 2017)**.

Table 5. Water physico-chemical parameters

Water parameters	**Values**
Temperature °C	26.8 ± 1.1
Dissolved oxygen mg/l	5.5 - 6.5 ± 0.4
pH	7.2 ± 0.3
Total ammonia nitrogen mg/l	0.022 ± 0.01
Nitrate mg/l	0.7 ±0.04
Nitrite mg/l	0.023±0.011

Growth performance of Nile tilapia

The present results showed that, the growth performance of monosex *Oreochromis niloticus* were improved when fed on the three supplemented diets comparedto the control one. The best values were recorded for fish fed diet 3(mixture of oil and powder) Table (6). These results may bedue to the rich phenolic compounds content in the orange peel which may enhance digestion and intestinal function. It is worthy to mention that, orange peel containshigh content of fibres and pectin that improve intestinal function and

health due to their physicochemical properties **(Chau *et al.*, 2005; Doan *et al.*, 2018)**. The present data revealed that the three forms of orange peel additives acted as a direct fish growth promoter. In addition, with respect to the survival rate, the present findings showed that they increased in all fish groups fed on experimental diets compared to the control.

Table 6. Growth performance of Nile tilapia fed the additives (Mean ± SE)

Growth parameter	Experimental diets			
	Control	Diet 1	Diet 2	Diet 3
Initial weight(g)	$17.5^{a} \pm 0.10$	$17.2^{a} \pm 0.12$	$16.9^{a} \pm 0.08$	$17.5^{a} \pm 0.13$
Final weight(g)	$91^{d} \pm 0.65$	$98.2^{c} \pm 1.07$	$104.1^{b} \pm 0.59$	$105.3^{a} \pm 0.54$
Body weight gain (g)	$73.5^{c} \pm 1.30$	$81.0^{b} \pm 0.78$	$87.2^{a} \pm 0.75$	$87.8^{a} \pm 1.04$
Daily weight gain(g/day)	$0.75^{c} \pm 0.01$	$0.83^{b} \pm 0.02$	$0.89^{a} \pm 0.11$	$0.90^{a} \pm 0.03$
Specific growth rate(%/day)	$1.68^{c} \pm 0.02$	$1.78^{b} \pm 0.01$	$1.85^{a} \pm 0.01$	$1.87^{a} \pm 0.03$
Survival rate (%)	94.5^{c}	96^{b}	98.0^{a}	97.5^{ab}

Feed utilization

The highest values of apparent protein digestibility, protein productive value, and protein efficiency ratio (78.32%, 41.26%, and 2.49, respectively) were found for fish fed on diet 3, accompanied by the optimal values of feed conversion ratio and feed consumed (1.58 and 138.92 g, respectively) (Table, 7).

The present results revealed that, the dietary inclusion of orange peel forms improved fish feed utilization, indicating that orange peel acts as fish appetizer.

Salem and Abdel-Ghany (2018) recorded that, orange peel powder in the tilapia diets (2%) enhanced the digestion and nutrient absorption. Additionaly, **Acar *et al.* (2015)** and **Gültepe (2018)** noticed that, the dietary inclusion of orange peel extracted oil enhanced growth performance of the Mozambique tilapia and Rainbow Trout.

Furthermore, **Goda (2008)** recorded that when the Nile tilapia (*Oreochromis niloticus*) fed diets supplemented with ginseng herb containing saponin, fish growth and diet utilization efficiency were enhanced. Remarkably, the sweet orange peel contains an ample amount of saponin **(Oluremi *et al.*, 2007)**.

Nevertheless, limited studies have been conducted on the effects of orange peel oil on animal growth parameters, thus this study is an initiative in this field.

Orange peels contain some important minerals and also huge amount of vitamin C. This advantage can be used in reducing supplemental minerals and vitamins in the diet, which could reduce the cost of feed production **(Nwanna *et al.*, 2011)**.

Table 7. Feed utilization parameters of fish fed additive (Mean ± SE)

Feed Utilization	Experimental Diets			
	Control	**Diet 1**	**Diet 2**	**Diet 3**
Feed Consumed (g)	$123.48^{d} \pm 0.11$	$130.82^{c} \pm 1.92$	$136.76^{b} \pm 1.28$	$138.92^{a} \pm 1.51$
Feed Conversion Ratio	$1.68^{c} \pm 0.05$	$1.62^{b} \pm 0.01$	$1.57^{a} \pm 0.03$	$1.58^{a} \pm 0.04$
Protein Efficiency Ratio	$2.33^{c} \pm 0.08$	$2.46^{b} \pm 0.10$	$2.50^{a} \pm 0.51$	$2.49^{a} \pm 0.53$
Protein Productive Value %	$33.55^{d} \pm 0.41$	$38.80^{b} \pm 0.37$	$37.11^{c} \pm 0.61$	$41.26^{a} \pm 0.54$
Apparent Digestibility %	$67.9^{d} \pm 0.62$	$76.2^{b} \pm 0.74$	$75.43^{c} \pm 0.52$	$78.32^{a} \pm 0.49$

Body chemical composition

Fig. (1) shows that, the highest protein and lowest lipid content were recorded for the fish fed diet 3 and diet 1, whereas the lowest protein accompanied with the highest lipid was recorded for those fed diet 2. **Townsley *et al.* (1953)** reported that, the orange peel contained the main amino acids, such as alanine, γ-aminobutyric acid, asparagine, aspartic acid, glutamic acid, leucine, phenylalanine, proline, serine, and valine.

The moisture content fluctuated insignificantly ($p>0.05$) through the three fish groups. Dry matter and ash content (Fig. 2) were increased for fish fed diets (1 & 3**)**. The high mineral content in the orange peel powderis assumed toincrease the mineralization in fish muscles (**Nwanna *et al.*, 2011)**.

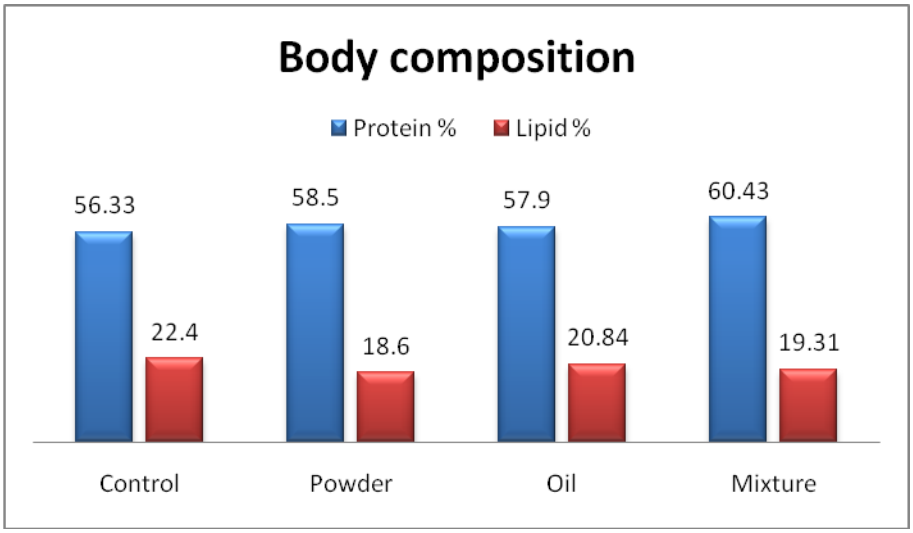

Fig. 1. Protein and lipid content in fish body

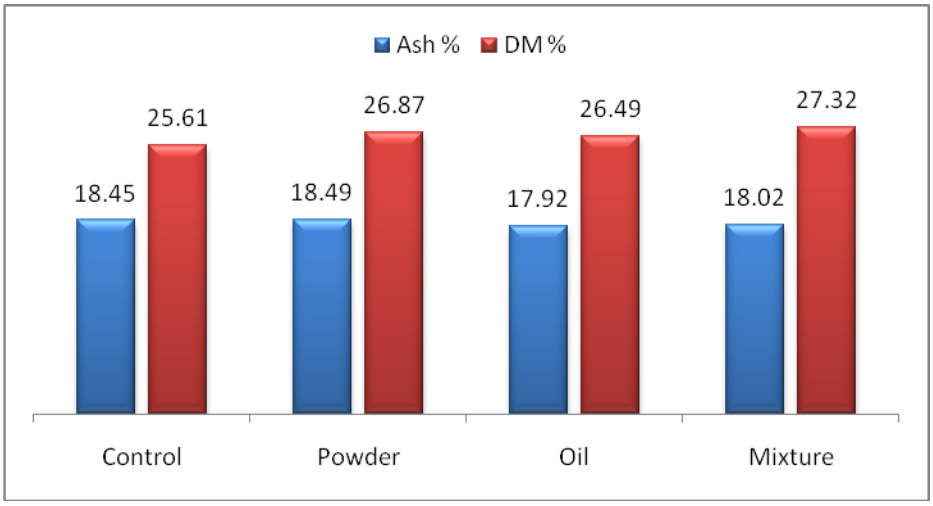

Fig. 2. Dry matter and ash content of fish body

Physiological parameters

a. Body indices

Body indices (Fig. 3) were affected by the experimental diets; the highest liver index and lowest gut index were recorded for those fed diet 2. These results may owe to the fibres contents, phenolic compounds, and pectin that may have improved the intestinal function and fish health **(Chau *et al.*, 2005; Doan *et al.*, 2018).**

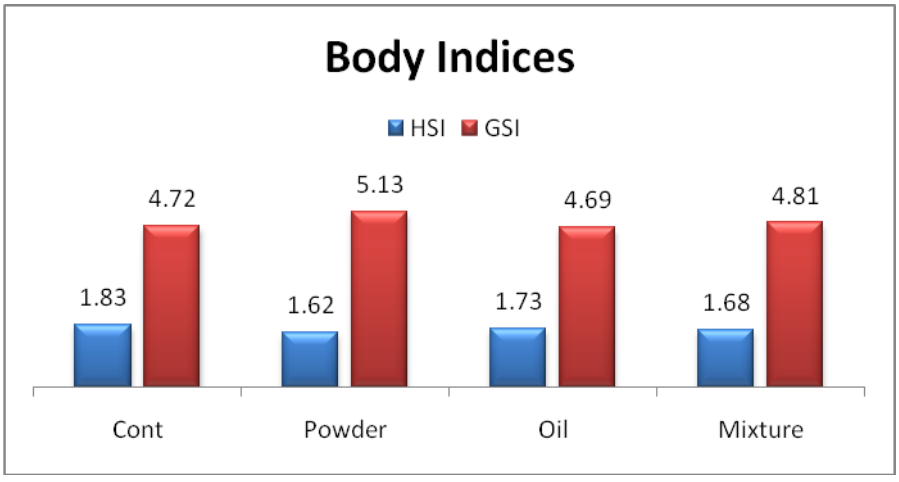

Fig. 3. Body indices of mono-sex tilapia fed orange peel

b. Biochemical parameters

Table (8) explains that, using orange peel additive enhanced plasma total protein and liver health (AST, and ALT) of the treated fish, and improved their immunity, while it helped in decreasing plasma glucose and cholesterol. The best plasma total protein value (3.78) was recorded for those fed the mixture.

Table 8. Biochemical parameters

Blood parameter	Experimental diets			
	Control	Diet 1	Diet 2	Diet 3
Glucose (mg/dl)	$96.1^{a} \pm 1.08$	$85.2^{d} \pm 0.91$	$90.3^{b} \pm 0.89$	$88.8^{c} \pm 0.99$
Cholesterol (mg/dl)	$126.0^{a} \pm 1.02$	$111.3^{c} \pm 1.10$	$114.5^{b} \pm 1.09$	$113.6^{bc} \pm 0.97$
Plasma Total Protein (g/dl)	$3.21^{c} \pm 0.02$	$3.70^{ab} \pm 0.04$	$3.61^{b} \pm 0.03$	$3.78^{a} \pm 0.05$
Aspartate Aminotransferase (AST) (U/L)	$136.4^{a} \pm 0.98$	$98.1^{c} \pm 1.04$	$101.5^{b} \pm 0.88$	$98.6^{c} \pm 0.79$
Alanine Aminotransferase (ALT) (U/L)	$69.3^{a} \pm 0.51$	$59.2^{d} \pm 0.49$	$66.5^{b} \pm 0.62$	$65.1^{c} \pm 0.38$
Immunoglobulin (IgM)	$11.2^{a} \pm 0.17$	$9.6^{b} \pm 0.13$	$9.36^{c} \pm 0.15$	$9.1^{d} \pm 0.12$
Immunoglobulin (IgG)	$137.6^{d} \pm 1.02$	$148.3^{c} \pm 1.06$	$151.5^{b} \pm 1.05$	$157.1^{a} \pm 1.08$

Terpstra *et al*. (2002) and **Figuerola *et al.* (2005)** suggested that, some physiological functions of the fibre content in dried orange peel may reduce blood cholesterol and glucose level. The previous authors added that, orange peel essential oil contains terpenes compounds, which are effective to reduce plasma cholesterol levels **(Dillard & German, 2000)**.

The present result cleared that, different forms of the additive (orange peel and oil) enhanced the enzyme activities and immunoglobulin (IgG) of the treated fish. This finding may be attributed to the presence of some bioactive components, such as phenols, amino acids, essential oils, pectin, carotenoids, flavonoids, and vitamin C which have positive biochemical functional effects as antioxidant agents **(Immanuel *et al.*, 2009; Hashemi & Davoodi, 2011; Assini *et al.*, 2013; Acar *et al.*, 2015; M'hiri *et al*., 2015; Yilmaz, 2019; Fadda & Raky, 2021; Raky *et al*., 2021).**

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