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**Effect of dietary supplementation of extracted jojoba meal on hematology, biochemical parameters and disease resistance in Nile tilapia (*Oreochromis niloticus*) infected by *Aeromonas hydrophila***

**Sayed A. Abdel-Aziz<sup>1</sup>, Ahmed A. Saeed<sup>1</sup>, Abdel-Aleim F. Abdel Aleim<sup>1</sup>, Somayah M. Awad<sup>2</sup> and Ismail A. Sarhan<sup>2</sup>**

1- Pharmacology Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

2- Central Laboratory for Aquaculture Research –Abbassa - Egypt

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**Abstract**

The activities of extracted jojoba meal were evaluated experimentally via using 240 *Oreochromis niloticus* that were distributed into 8 equal groups (each of 3 replicates), Groups I (non infected) and II (infected) fed on balanced diet without treatment, groups III (non infected) and VI (infected) supplemented with extracted jojoba meal by (0.5g/kg) on balanced diet, groups IV (non infected) and VII (infected) supplemented with extracted jojoba meal by (1g/kg) on balanced diet, groups V (non infected) and VIII (infected) supplemented with extracted jojoba meal by 2g/kg on balanced diet. Hematological, Biochemical parameters, Mortality rate were determined. Blood samples were collected from the experimented fish after 90 days from the beginning of the experiment to measure Red blood cell (RBCS) count, Hemoglobin (Hb), Hematocrit (PCV) value, White blood cell (WBCS) count, Total protein, Albumin, Globulin and A/G ratio. The protective effect of the jojoba meal was evaluated via pathogenic test using pathogenic *A. hydrophila*. Result revealed that mortality rate (%) were significantly decreased in groups supplemented with Extracted jojoba meal (G III, G IV and G V) compared with control (Group I). The RBCS count, Hb, PCV value, WBCS count, Total protein, Albumin and Globulin showed significant increase in all supplemented groups (G III, G IV, G V, G VI, G VII, G VIII) compared with control (G I and II). On the other hand, a Significant decrease in A/G ratio has been observed in all supplemented groups (G III, G IV, G V, G VI, G VII, G VIII) compared with control (G

I and II). therefore, supplementation diet with extracted jojoba meal improved the hematological parameters, general health condition and decrease mortality rate of *O. niloticus* in aquaculture.

**Keywords:** Jojoba, *O. niloticus*, *A. hydrophila*, Hematology, Biochemical.

### **Introduction**

Plants have been a very good source of medicinal compounds that has continued to play a dominant role in the maintenance of human health because medicinal plants represent a rich source of antimicrobial agents, they are also a rich sources of bioactive compounds Mariita *et al.*,2011.

There are thousands of species of medicinal plants used globally for the cure of different infections. These plants are used as antimicrobial agents and several works have been carried out to find out its scientific basis. In traditional field medicine, herbs have been used as immune stimulants for thousands of years Tan and Vanitha 2004. These herbs contain many types of active components, like polysaccharides, alkaloids or flavonoids. The immune stimulating activity of herbal components have been most widely studied in mice, chickens, human and fish Shan *et al*, 1999, Cao and lin 2003 and lin and Zhang 2004.

There is a growing interest in using medicinal herbs as immune stimulants in aquaculture, especially Nile tilapia (*Oreochromis niloticus*) which was the predominant and most commonly cultured species among tilapias in many countries around the world Mehrim ,2009, including Egypt Zaki *et al* 2011. They are recognized as the species of choice for intensive aquaculture and are likely to become the most important cultured fish in the world Fitzsimmons 2008 and Harikrishnan *et al*, 2011.

Enhancement of the immune system seems to be the most promising method of preventing fish diseases. In which motile Aeromonads, especially *Aeromonas hydrophila* is known to be one of the most important bacteria associated with diseases in freshwater fishes including Nile tilapia Marzouk *et al* 2010 and Parker *et al.*, 2011. In response, large amounts of antibiotics are traditionally used by fish farmers for disease prevention and control. However, the massive use of antibiotics in aquaculture may be harmful to the environment and human health Harikrishnan *et al*, 2011.

The wide and frequent application of antibiotics in aquaculture systems result in residual antibiotics in the environment that cause the increase in the emergence of antibiotic resistance in aquatic bacteria Vivekanandhan *et al.*, 2002. Therefore, medicinal plant represents safe alternatives to the

use of antibiotics, which are gaining importance in many countries Buller 2004 and Aly *et al.*,2008.

Medicinal plant provides a promising alternative approach for controlling fish diseases, which control pathogens through a variety of mechanisms, especially by enhancing immune system of fish. stimulate immune system of fish by medicinal plant act by enhance the innate (or non-specific) immune response, Secombes and Fletcher, 1992, Magnadótti., 2006.

The Jojoba meal that left over after seed's oil extraction contains from 26% to 33% crude protein Ashour *et al* 2013 as well as carbohydrate, fiber minerals, vitamins and anti-microbial agent Richard King *et al.*,2005

However, Jojoba meal is underutilized because it contains four anti-nutritional compounds representing 11-15% of the meal and collectively known as simmondsins, that are found unpalatable and have adverse effects on animals, Miwa, 1984, Richard King *et al* 2005 and Sharma, and Singh 2011. The jojoba meal could convert into raw material for animal feed and simultaneously, decrease the negative impact on the environment. Jojoba meal, as a by-product of jojoba seeds squeeze, is a promising feed stuff after being detoxified. This plant extract (Jojoba meal) has been reported to be useful as a dietary supplement as food additive, a medical food and as a therapeutic agent Richard King *et al* 2005.

Different extracts from jojoba plant are widely used in many folk medicinal uses. Most of the coming work was directed only on the extract of jojoba seed oil (jojoba meal) of its medicinally important plant in Aquaculture Ashour *et al* 2013

This study was designed to evaluate the efficacy of extracted jojoba meal and its effect on general health and mortality rate of Nile tilapia, *O. niloticus*.

## **Material and Methods**

### **Jojoba meal**

Jojoba meal obtained from The Egyptian Natural Oil Company, Egypt. All the dietary ingredients and additives were obtained from the local market and all ingredients and additives were milled and mixed, then pressed by manufacturing machine.

### **Treating of jojoba meal**

It is recommended that Simmondsin should be previously removed from the Jojoba meal after oil squeeze. Accordingly, Jojoba meal was

treated by heating at 100°C for 3 hours or boiled for one hour. Abd El-Rahman *et al.*, 2006.

### **Preparation of hydro-alcoholic extract of Treated jojoba meal**

The dried treated jojoba meal was grounded into fine coarse powder by hand and mortar. The treated jojoba meal was soaked in ethanol 95% (II): water I: 1 at 33c for 24h. The mixture was subjected to filtration using I filter paper. After that the solvent was evaporated using rotary evaporator then the extract was lyophilized and stored at -4°C in dark bottle Prabsattroo *et al* 2012.

### **Biological agent (bacterial agent)**

*Aeromonas hydrophila*, the identified *Aeromonas hydrophila* isolate with standard known biochemical and pathogenicity profiles used in the experimental infection was kindly obtained from Microbiological Archive of the Microbiology Department of Central laboratory for Aquaculture Research.

### **Fish**

A total of 240 apparently healthy mono sex Nile tilapia (*O. niloticus*) were collected from the World Fish Center, Abbassa, Egypt (mean individual initial weight 40±5 g). Fishes were divided into 8 equal groups (30 fish each) distributed into 3 replicates (10 fish each). They were reared in glass aquaria (50× 60×120 cm), fed on a balanced commercial diet at a ratio of 5% of body weight per day. The water was partially renewed daily, the temperature maintained at 25±1 °C. Fishes were maintained in the aquaria for couple weeks for adaptation.

### **Basal diet**

Pellets (0.5 cm) were prepared from locally available ingredients using a pellet machine (CPM California Pellet mill, San Francisco, CA, USA). The ingredients as illustrated in Table 1 were mixed mechanically with a horizontal mixer (Hobarts model D320T, Troy, OH, USA) at a low speed for 30 min after crushing the corn to a size of 0.1 mm using a Thomas-Willey laboratory Mill Model 4. Then, oil was added gradually to ensure an even distribution of the ingredients with an increase in the mixer speed for 5 min, during that time 600 ml water was added. The obtained pellets were dried at room temperature for 24 h. A diet was formulated to contain 3 formulated ration, 1<sup>st</sup> applied by addition of 0.5g/kg to the basal diet, 2<sup>nd</sup> applied by addition of 1 g/kg to basal ration and 3<sup>rd</sup> applied by addition of 2 g /kg to basal diet, the required diet was prepared biweekly and stored in a refrigerator at (4 °C) for daily use.

**Table 1: Experimental diets composition/kg %**

Soya bean meal	20%
Fish meal	12%
Corn	38%
Poultry by product	12%
Palm oil	5.5%
Premix	1.5%
Wheat flour	10%
Di calcium phosphate	1%
<b>Chemical composition</b>	<b>% (DM)basis</b>
Dry matter	90.42
Crude protein	30.158
Ether extract	4.75
Ashes %	12.45
Carbohydrate	52.22
Gross energy (GE) (al/100 g DM)	431.34
Protein/energy (P/E) ratio (mg CP/KcalGE)	70.1

**Experimental design:**

The current study was conducted for 3 months (90 days) to evaluate the efficacy of extracted jojoba meal on Nile tilapia (*O. niloticus*) aquaculture.

Start from the beginning of experimental till 90 days of feeding and consist of 240 fish of *O. niloticus* were divided into 8 equal groups, each group consist of 3 replicates illustrated in table 2.

**Table, (2): Experimental design.**

groups	Number of fish	Treatment	Infection
G I	30	Fed on a basal diet without treatment (control negative)	Non infected
G II	30	Fed on a basal diet without treatment (control positive)	Infected
G III	30	0.5g/kg extracted jojoba meal supplemented basal diet	Non infected
G IV	30	1g/ kg extracted jojoba meal supplemented basal diet	Non infected
G V	30	2g/ kg extracted jojoba meal supplemented basal diet	Non infected
G VI	30	0.5g/kg extracted jojoba meal supplemented basal diet	Infected
G VII	30	1g/kg extracted jojoba meal supplemented basal diet	Infected
VIII	30	2g/kg extracted jojoba meal supplemented basal diet	Infected

G II, G VI, G VII, G VIII of Fish were artificially infected by an intra peritoneal (I/P) route with 0.5 ml by sub lethal dose of microbial suspension of pathogenic *A. hydrophila* containing  $10^8$  bacteria ml at 75 days of feeding and left for 14 days till end of experiment (90 days) then start collect blood sample after 24 from end of experimental (at day 91).

Blood samples were collected from Fishes for evaluated for hematological parameters and serum sample for bio chemical analysis.

#### **Blood sampling:**

Ten fish were randomly collected from each treatment and the control. The fishes were anesthetized by immersion in water containing 0.1ppm clove oil. Whole blood (0.5 ml) was collected from the caudal blood vessels of each fish using syringes (1-ml) and 27-gauge needles that were

rinsed with heparin (15 unit/m), to determine the RBCS count, Hb value, hematocrit (PCV) values and WBCS count other blood sample were centrifuged to obtain serum to determine total protein, Albumin, Globulin and A/G ratio

#### **Mortality rate:**

Fish from all groups were put under observation after injected by pathogenic strain of *A. hydrophila* to evaluate the mortality rate which recorded along the period of pathogenicity test (15 days).

#### **Hematological and Biochemical analysis:**

##### **Total RBCS, WBCS and differential leukocyte count:**

The RBCS and total WBCS count was determined using a hemacytometer with Neubauer counting chamber, Natt and Herrk's solution as diluting fluid and 1: 100 diluted blood according to the method described by Stoskopf, 1993.

##### **Hematocrit level:**

Hematocrit value was measured according to Smith., (1967).

##### **Total protein:**

Serum total protein was measured by the biuret method using a commercially available kit Koller, 1984.

##### **Albumin determination:**

Serum albumin level was determined according to method described by Tietz, 1994.

##### **Calculation of globulin level and A/G ratio:**

Globulin value was obtained by subtracting albumin from total protein, while A/G ratio was calculated by dividing albumin level by globulin level.

##### **Pathogenicity test**

Ten fish from each treatment of (G I, G III, G IV, G V) were randomly collected after end of experimental study (after 90 days of feeding) and reared in glass aquaria. They were clinically examined and blood samples were tested to ensure that they were free from bacterial infection. The previous groups were submission to pathogenicity test at the end of experimental study (90 days). The pathogenic bacterium was obtained as a pathogenic *A. hydrophila* strain that was previously isolated from *O. niloticus* and tested for pathogenicity in Central laboratory for Aquaculture Research (Egypt). A suspension of *A. hydrophila* was prepared by culturing in Tryptic soy agar for 24 h., then collected, washed, and suspended in sterile saline 0.85% and counted using McFarland standard

tubes. Fishes were then artificially infected by an intra peritoneal I/P injection with 0.5 ml of microbial suspension of pathogenic *A. hydrophila* containing  $10^8$  bacteria ml. The challenged fish were observed for 15 days after infection and mortality rate recorded.

#### **Statically analysis:**

Statistical analysis was performed using the one-way analysis of variance (ANOVA) and Dauncan Multiple Range Test was carried to determine the differences between treatment (mean at significance level of  $p < 0.05$ ). Standard errors were also estimated. All analysis was run on the computer using the SAS program (SAS Institute Cary, North Carolina, USA) SAS, 2005.

#### **Results:**

##### **Effect of the extracted jojoba meal on *O. niloticus***

##### **RBCS count, Hb, PCV:**

It was clearly evident from table 3 that the addition of extraction of jojoba meal by concentration (0.5g/kg (G III, G VI) (1 g/kg (G IV, G VII) and 2g/kg (G V, G VIII) to basal diet of *O. niloticus* for successive 90 days revealed the following result:

Non infected group G III (0.5g/kg) G IV (1 g/kg) and G V(2g/kg) showed significant increase in RBCS count, Hb and PCV value compared with control negative (non infected) (G I).

infected group G VI (0.5g/kg), G VII (1g/kg) and G VIII (2g/kg) showed significant increase in RBCS count, Hb and PCV value compared with control positive (infected) (G II).

**Table 3: The effect of jojoba meal extract ((0.5g, 1g and 2g) kg) on non infected and infected *O. niloticus* with *A. hydrophila* on RBCS count ( $10^6/\mu\text{l}$ ), Hg g/dl and HCT % mean  $\pm 5$  N =10**

Groups	After 90 days of feeding		
	RBCS count $\times(10^6/\text{mm}^3)$	Hb gm % value	PCV % Value
G I control N	$0.74 \pm 0.036$ c	$5.75 \pm 0.098$ c	$12.25 \pm 0.36$ d
G II control P	$0.36 \pm 0.012$ c	$3.85 \pm 0.12$ d	$1.373 \pm 0.039$ ab
G III non N 0.5g/kg	$1.84 \pm 0.036$ a	$9.85 \pm 0.098$ a	$16.39 \pm 0.40$ ab
G IV N 1g/kg	$1.88 \pm 0.036$ a	$9.9 \pm 0.098$ a	$15.55 \pm 0.40$ a
G V N 2g/kg	$1.75 \pm 0.036$ b	$9.275 \pm 0.098$ b	$16.72 \pm 0.40$ ab
G VI P 0.5g/kg	$1.58 \pm 0.039$ b	$8.05 \pm 0.13$ b	$17.97 \pm 0.36$ b
G VII P 1 g/kg	$1.44 \pm 0.039$ a	$8.56 \pm 0.13$ a	$18.15 \pm 0.36$ a
VIII P 2g/kg	$1.45 \pm 0.039$ c	$8.42 \pm 0.13$ cd	$17.37 \pm 0.36$ c

Mean value carrying different letters or superscript in the same column show significant at  $P < 0.05$ .

### WBCS Count and its differential:

It was clearly evident from table 4 that the addition of extraction of jojoba meal by concentration (0.5 g/kg (G III, G VI) (1 g/kg (G IV, G VII) and 2g/kg (G V, G VIII) to basal diet of *O. niloticus* for successive 90 days revealed the following result:

Non infected group G III(0.5g/kg) G IV (1 g/kg) and G V(2g/kg) showed significant increase in: WBCS, lymphocyte, Monocyte and Granulocyte count compared with control negative (non infected) (G I).

infected group G VI(0.5g/kg), G VII (1 g/kg) and G VIII (2g/kg) showed significant increase in WBCS, lymphocyte, Monocyte and Granulocyte count compared with control positive (infected) (G II).

**Table 4: The effect of jojoba meal extract ((0.5g, 1g and 2g) kg) on non-infected and infected *O. niloticus* with *A. hydrophila* on WBCS count ( $10^3/\mu\text{l}$ ), lymphocyte ( $10^3/\mu\text{l}$ ), monocyte ( $10^3/\mu\text{l}$ ) and granulocyte ( $10^3/\mu\text{l}$ ) mean  $\pm$  5 N =10**

groups	After 90 days of feeding			
	WBCS count ( $10^3/\mu\text{l}$ )	lymphocyte ( $10^3/\mu\text{l}$ ),	monocyte ( $10^3/\mu\text{l}$ )	granulocyte ( $10^3/\mu\text{l}$ )
G I control N	22.08 $\pm$ 1.06b	18.85 $\pm$ 0.84c	2.11 $\pm$ 0.39 c	1.12 $\pm$ 0.31c
G II control P	35.78 $\pm$ 0.72 d	24.74 $\pm$ 0.53 c	9.50 $\pm$ 0.56 bc	1.54 $\pm$ 0.44 c
G III N 0.5g/kg	48.92 $\pm$ 0.94 a	34.35 $\pm$ 0.66 a	10.31 $\pm$ .75 ab	4.26 $\pm$ 0.41 a
G IV N 1g/kg	49.055 $\pm$ 0.9 ab	34.25 $\pm$ 0.66 a	10.76 $\pm$ 0.75 b	4.04 $\pm$ 0.41 a
G V N 2g/kg	48.20 $\pm$ 0.94 c	35.32 $\pm$ 0.66 a	8.17 $\pm$ 0.75 c	4.7125 $\pm$ 0.41 a
G VI P 0.5g/kg	55.87 $\pm$ 0.72 a	36.90 $\pm$ 0.53 ab	12.64 $\pm$ 0.56 a	6.33 $\pm$ 0.44 ab
G VII P 1g/kg	54.72 $\pm$ 0.72 bc	36.315 $\pm$ 0.53 b	12.42 $\pm$ 0.56 bc	5.99 $\pm$ 0.44 ab
VIII P2g/kg	54.17 $\pm$ 0.72 c	35.95 $\pm$ 0.53 b	12.745 $\pm$ 0.56 c	5.48 $\pm$ 0.44 b

Mean value carrying different letters or superscript in the same column show significant at  $P < 0.05$ .

### Biochemical Parameters

#### Serum protein, Albumin, Globulin and A/G ratio:

It was clearly evident from table 5 that the addition of extraction of jojoba meal by concentration (0.5g/kg, 1 g/kg and 2g/kg) to (G III, G VI) (G IV, G VII) and (G V, G VIII) receptively to basal diet of *O. niloticus* for successive 90 days revealed the following result:

Non infected group G III, G IV and G V showed significant increase in in serum protein, albumin, globulin value and decrease in A/G ratio compared with control negative (non-infected) (G I),

Infected group G VI, G VII and G VIII showed significant increase in serum protein, albumin, globulin value and decrease in A/G ratio compared with control positive (infected, G II).

**Table 5: The effect of jojoba meal extract ((0.5g, 1g and 2g) kg) on non-infected and infected *O. niloticus* with *A. hydrophila* on total serum protein g/dl, Albumin value (g/dl), Globulin value (g/dl) and A/G Ratio mean  $\pm$ 5 N =10**

groups	After 90 days of feeding			
	total serum protein g/dl	Albumin value g/dl	Globulin value g/dl	A/G Ratio
G I control N	0.59 $\pm$ 0.01 d	0.47 $\pm$ 0.0079c	0.12 $\pm$ 2.5 a	3.9 $\pm$ 0.04a
G II control P	0.72 $\pm$ 0.005 d	0.42 $\pm$ 0.0071 d	0.30 $\pm$ 0.036 b	1.4 $\pm$ 0.024a
G III N 0.5g/kg	1.36 $\pm$ 0.005 a	0.73 $\pm$ 0.0071a	0.63 $\pm$ 0.036a	1.15 $\pm$ 0.024b
G IV N 1g/kg	1.35 $\pm$ 0.005ab	0.73 $\pm$ 0.0071a	0.62 $\pm$ .036 a	1.17 $\pm$ 0.024b
G V N 2g/kg	1.36 0.005ab	0.73 $\pm$ 0.007c	0.63 $\pm$ 0.036 a	1.15 $\pm$ 0.024c
G VI P0.5g/kg	1.42 $\pm$ 0.005 a	0.74 $\pm$ 0.0071a	0.68 $\pm$ 0.036a	1.08 $\pm$ 0.024b
G VII P 1 g/kg	1.43 $\pm$ 0.005ab	0.75 $\pm$ 0.0071a	0.68 $\pm$ .036 a	1.10 $\pm$ 0.024b
VIII P 2g/kg	1.43 $\pm$ 0.005ab	0.73 $\pm$ 0.007c	0.70 $\pm$ 0.036 a	1.04 $\pm$ 0.024c

**Mean value carrying different letters or superscript in the same column show significant at P<0.05**

#### **Pathogenicity test:**

Results in Table (6) showed that, Groups G VI, G VII and G VIII which received jojoba meal extracted in diet show significant decrease in mortality rate compared with G II infected control positive.

No significant difference in mortality recorded between treated non infected G III, G IV and G V compared to G I control (non-infected non treated).

**Table 6: The effect of jojoba meal extract a non-infected *O.niloticus* after 90 days of feeding injected with pathogenic *A. hydrophila* on Mortality rate mean  $\pm 5$  N =30**

Peroids groups	N of f	0 d	1 <sup>st</sup> d	2 <sup>nd</sup> d	3 d	4 d	5d	6d	7d	8d	9d	10d	11d	12d	13d	14d	MR %
G I control N	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0%
G II control P	30	5	3	2	1	0	1	1	1	0	0	0	0	0	0	1	50%
G III N 0.5g/kg	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0%
G IV N 1g/kg	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0%
G V N 2g/kg	30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0%
G VI P 0.5g/kg	30	3	1	1	0	0	0	0	1	0	0	1	0	1	0	1	30%
G VII P 1 g/kg	30	3	2	0	0	1	1	0	0	0	0	1	0	0	1	0	30%
G VIII P 2g/kg	30	2	2	1	1	0	1	0	1	0	0	0	1	0	0	0	30%

MR: Mortality rate

N: number

F: fish

D: day

## Discussion

The results showed that ethanolic extract of jojoba meal after treatment appears to improve the haemobiotic system after treatment. There was significant increase in RBCS count, Hg, PCV, WBCS count and its differential may be due to increase synthesis of blood cells or probably due to increase minerals and vitamins which help in of blood cells synthesis by saponin which had been earlier reported to be present in the jojoba plant Shrestha *et al.*, 2002.

These results were in full agreement with Saleh and Toutou ,2015 who studied the effect of replacing soybean meal (SBM) by treated, either by heat (Heated Jojoba Meal) or by boiling (Boiled JM), Jojoba meal (JM), to eliminate the negative effects of anti-nutritional factors, at 10, 20 and 30% replacing levels in diets of sea bream. Results of fish blood parameters indicate significant increase in RBCS count, Hg value and HCT value in fish fed 10 and 20% HJM and 20% BJM.

Similarly, Talpur, 2014 who studied the phenolic compound to determine the influence of these compounds on general health of fish (Asian sea bass). they reported that the potential effects of phenolic compound on the growth performance, immune response and protection against *Vibrio harveyi* infection of sea breams, therapeutics concentrations of these compounds caused a dose dependent increase in hemoglobin values coupled with increase in hematocrit values and red blood cell counts are an obvious indication of good health of fish. The total white blood cell counts and the differential white blood cell counts were increased especially the lymphocytes, in experimental fish when compared with control. The result from this study reveals high effect of plant containing phenolic compounds on general health of fish in increasing level of erythrocytes, leucocytes, haematocrit and haemoglobin.

Regarding to biochemical parameters, at the end of experiment, significant increase of total protein, albumin and globulin and decrease in A/G ratio in treated groups was observed when compared to control group ( $p \leq 0.05$ ), the increase in total protein and albumin might be due to increase in the process of protein synthesis in liver due to demand during stress produced due to infection, also the significant increase of globulin level may be attributed to increase the enhancement of innate immunity response and increase production of antibody, Riche *et al.*, 2007.

Alterations in total serum protein, albumin and globulin concentration have been used as a broad clinical indicator of fish health status Wiegertjes *et al.*, 1996.

Theses result of biochemical parameters agree with Talpur, 2014 and Saleh and Toutou 2015, **who** applied study on the phenolic compound to determine the influence of these compounds on general health of fish, For total serum protein, albumin and globulin. The derived resulted relived a high increase in Serum protein, albumin and globulin in experimental fish when compared with control, also the result from this study reveals high effect of plant containing phenolic compounds on general health of fish. Finally, the results showed obvious incensement in Serum total protein, albumin and globulin levels compared with the effects when soybean meal was not replaced by jojoba meal.

Our study supported by Ramstead *et al.*, 2012 who proved that immune response is one of the most studied effects of plant phenolic compounds which extracted from many plant sources, the experiments being carried out both in vitro and in vivo on different species: humans, fish and bovine. In this respect, it was showed that polyphenol compound isolated from many plant sources, is known to increase level of serum protein, albumin, globulin, activate myeloid cells and stimulate innate lymphocytes, including bovine and human T cells and NK cell

Our study revealed that all treated non infected groups which injected with pathogenic *Aermonas hydrophila* showed low mortality rate when compared with infected control groups.

These results agreement with Talpur, 2014 who studied the potential effects of phenolic compound on the growth performance, immune response and protection against *Vibrio Harvey infection* in Asian sea bass *lates calcarifer*. The results showed that phenolic compound diet in feed led to reduced mortalities and significantly improved survival, weight gain and feed conversion ratio for treated groups over the control. Phenolic compound diet led to the enhancement of the immunity of *l. calcarifer* against infection. likewise, the impact of phenolic compound has shown effective therapeutic results to consider incorporating the plant containing the compound in fish feed to replace the use of antibiotics or therapeutics for sustainable aquaculture.

Similarly, Sivaram *et al.*, 2004 reported that some natural plant products have anti stress, growth promotion, survival growth, appetite stimulation, tonic, immune stimulation, aphrodisiac and antimicrobial properties in finfish and shrimp larvae culture due to the presence of active principle components such as alkaloids, flavanoids, pigments, phenolics, terpenoids, steroids, and essential oils.

Our results supported by Jian and Wu, 2003 who studied the effect of Feeding of a traditional plant medicine derived from Astragalus root Chinese angelica and Simmondsia chinensis root to large yellow croaker (*Pseudosciaena crocea*) for 30 days enhanced lysozyme and complement activities and NBT-positive cells as well as survival rate (93.3%) compared with cumulative mortality of 75% in control fish infected with *Vibrio alginolyticus*.

Our results supported by logambal *et al.*, 2000 showed that rainbow trout with feeding of 1% aqueous extract powder of *Simmondsia chinensis* roots (jojoba roots) for 3 weeks showed enhanced phagocytosis, respiratory burst activity, total protein level, increase survival rate and decrease in mortality rate compared to the control group

Our results coincided with Ardo *et al.*, 2008 who reported that Feeding tilapia with two extracted medicinal herbs (phenolic compound and flavonoids) alone or in combination significantly reduced the mortality following *Aeromonas hydrophila* infection. The lowest mortality was observed in the group fed with the combination of both extraction and boron Combination of the extraction and boron.

### **Conclusion**

It could be concluded from obtained results that, the addition of jojoba meal extracted to the feed of fish improve heambiotic system, general health of fish and increase disease resistant and survival rate against bacterial infection. So we advise it's better to the farmer and different company to use of jojoba meal as part of feeding of fish.

### **Conflict of interest**

**The authors have no conflict of interest to declare**

### **REFERENCE**

- Abdel-allah Dad Talpur (2014).** *phenolic compound* as feed additive enhanced growth performance, survival, immune response and disease resistance of Asian seabass, *latescalcarifer* (Bloch) against *Vibrio harveyi* infection, Aquaculture pages : 71 - 78 Volumes 420–421, 15 January.
- A. E. Abd El- Rahman, A. A., Abd El- Aleem, and I. M. I El (2006).**“Elimination of toxic compounds and nutritional evaluation of jojoba meal proteins” EJBMB 24 (April), 583- 602.
- Aly, S. M.; Abd El-Rahman, A. M.; John, G and Mohamed, M. F. (2008).** Characterization of Some Bacteria Isolated from *Oreochromis niloticus* and their Potential Use as Probiotics. Aquaculture, 277, 1-6.

- Ashour M. I., Ayoub N. A., Singab A. N. B., and AlAzizi M. M., (2013).** *Simmondsiachinensis* (Jojoba): A comprehensive pharmacognostic study." *Journal of Pharmacognosy and Phytochemistry*, vol. 2, no. 2.
- Ardol, Yin G, Xu P, Váradi, Szigeti G, Jeney Z, Jeney G. (2008).** Chinese herbs (*Astragalus membranaceus* and *Ionicera japonica*) and boron enhance the non-specific immune response of Nile tilapia (*Oreochromis niloticus*) and resistance against *Aeromonas hydrophila*. *Aquaculture* 275: 26-33.
- Buller, N. B. (2004).** *Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual*. CABI Publishing, Cambridge.
- Cao, I.Z. and lin, Z.B., (2003).** Regulatory effect of Ganoderma lucidum, polysaccharides on cytotoxic T-lymphocytes induced by dendritic cells in vitro. *ActaPharmacologicaSinica* 24 (4), 312–326.
- Fitzsimmons, K. (2006).** Prospect and potential for global production. Pages 51- 72 in C. lim and C.D. Webster, Editors. *Tilapia: Biology, Culture and Nutrition*. The Haworth Press, Inc., Binghamton, New York, NY.
- Harikrishnan R, Kim, JS, Kim, MC, Balasundaram C and Heo MS. (2011).** Hericium erinaceum enriched diets enhance the immune response in *Paralichthys olivaceus* and protect from *Philasterides dicentrarchi* infection. *Aquaculture*.
- Jian J, Wu Z. (2003).** Effects of traditional Chinese medicine on nonspecific immunity and disease resistance of large yellow croaker, *Pseudoscia enacrocea* (Richardson). *Aquaculture* 218(1-4): 1-9.
- Koller, A. (1984).** Total serum protein. *Clin.Chem.*The C.V. Mosby Co. St. louis, Toronto. Princeton, 418: 1316-1324.155 .
- lin, Z.B., Zhang, H.N., (2004).** Anti-tumor and immune regulatory activities of Ganoderma lucidum and its possible mechanisms. *ActaPharmacologica, Sinica* 25 (11), 1387–1395.
- logambal SM and Michael RD. (2000).** Immuno stimulatory effect of azadirachtin in *Oreochromis mossambicus* (Peters). *Ind J Experiment Biol* 38: 1092-1096.
- Magnadóttir, B., (2006).** Innate immunity of fish (overview). *Fish and Shellfish Immunology* 20, 137–151.
- Mariita R. M., Ogal C. K. P. O., Oguge N. O., and Okemo P. O., (2011).** Methanol extract of three medicinal plants from Samburu in northern Kenya show significant Antimycobacterial, antibacterial and antifungal properties," *Research journal of medicinal plants*, vol.5, no. 1, 54-64.

- Marzouk, M. S.; Abd ElAziz ,M. A.; Moustafa, M. and Abotorkia, S. (2010).** Alarming the critical times for occurrence of important bacterial problems in cultured *Oreochromis niloticus* under semi-intensive system in Sharkiaprovince. Assiut Vet. Med. J., 56 (126): 58-80.
- 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: 261-273.
- Miwa T.K., (1984).** Structural determination and uses of jojoba oil J Am Oil Chem. Soc, 1984. vol.61, no. 2, pp. 407- 410, 1984.
- Norhan E. Saleh, Mohamed M. Toutou (2015).** Effects of Replacing Soybean Meal with Jojoba Meal in Sea Bream (*Sparusaurata*) Diets on Fish Performance. American Journal of life Sciences. Special Issue: New Horizons in Basic and Applied Zoological Research. Vol. 3, No. 6-1, 2015, pp. 62-68.
- Parker, J. I. and Shaw, J. G. (2011).** *Aeromonas spp.* clinical microbiology and disease. J Infect., 62:109-118.
- Prabsattroo T, Wattanathorn J, Iamsa-ard S, et al. Am J Neurosci, (2012).** “Methanol extract of three medicinal plants from Samburuinnorthern Kenya show significant Anti myco bacterial, antibacterial and antifungal properties,3(1):17–24.
- Ramstead A, Schepetkin I, Quinn M and Jutila M. Oenothien B, (2012).** A cyclic dimericellagitannin isolated from *Epilobiumangustifolium*, enhances IFN $\gamma$  production by lymphocytes. PloS One. 2012;7(11):e50546. DOI: 10.1371/journal.pone.0050546.
- Richard King T., Samuelleonard T., Frank louis J. Jr. and Richard M. Jr. (2005).** “Compounds for altering food intake in humans”, United Statesm Patent: 6,852,342; Issued: February 8 (2005), Avoca, Inc. (Merryhill,NC); ChromaDex, Inc. (Santa Ana, CA), Appl. No.: 108036.
- Riche M. (2007).** Analysis of refractometry for determining totalserum protein in hybrid striped bass (*Moronechrysops  $\times$  *M.saxatilis*) at various salinities. Aquaculture 264, 279–284.*
- SAS Institute INC (2005).** The statistical analysis system for windows 6-12 Ed. Cary N.C . USA. Vol., 7.Edited by cooper S, New York, and Academic Press.
- Secombes, C.J., Fletcher, T.C., (1992).** The role of phagocytes in the protective mechanisms of fish. Annual Review of Fish Diseases 2, 58–71.

- Shan, B.E., Yoshida, Y., Sugiura, T and Yamashita, U., (1999).** Stimulating activity of Chinese medicinal herbs on human lymphocytes in vitro. *International Journal of Immunopharmacology* 21, 149–159.
- Sharma S. K., and Singh A.P. (2011).** “Pharmacognostical evaluation of roots of *Simmondsiachinensis* Schneider,” *International Journal of Pharmaceutical Sciences and Drug Research*, vol.3, no.4, pp. 323 -326.
- Shrestha MK, Peri I, Smirnoff P, Birk Y and Golan-Goldhirsh (2002).** A. Jojoba seed meal proteins associated with proteolytic and protease inhibitory activities. *J Agric Food Chem* . 2002;50(20):5670-5675.
- Sivaram V, Babu MM, Citarasu T, Immanuel G, Murugadass S and Marian MP. (2004).** Growth and immune response of juvenile greasy groupers (*Epinephelustauvina*) fed with herbal antibacterial active principle supplemented diets against *Vibrio harveyi* infections. *Aquaculture* 237: 9-20
- Smith, C. E. (1967).** Hematological change in coho salmon fed folic acid different diet" *Journal of the Fisheries Research Board of Canada*. 25,(151-159).
- Stoskopf M. (1993).** Fish medicine [Chapter4] .WB .Saunders Company, Harcourt Brace Jovanovich ,Inc.
- Tan, B.K.H and Vanitha, J., (2004).** Immunomodulatory and antimicrobial effect of some traditional Chinese medicinal herbs. *Current Medical Chemistry* 11, 1423–1430.
- Tietz, T. (1994).** Textbook of clinical chemistry 2<sup>nd</sup> ed. Saunder WB. Company. Philadephia.
- Vivekanandhan, G.; Savithamani, K.; Hatha, A. A. M. and lakshmanaperumalsamy, P. (2002).** Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India. *International Journal of Food Microbiology*, 76, 165-168.
- Wiegertjes, G.F., Stet R.J.M., Parmentier H.K. and Van Muiswinkel W.B. (1996).** Immuno-genetics of Disease Resistance in Fish: A Comparative Approach". *Develop. Compar. Immun.* 20: 365-381.
- Zaki, M. M.; Eissa A. E. and Saeid, S. (2011).** Assessment of the immune status in Nile Tilapia (*O.niloticus*) experimentally challenged with toxogenic and septicemic bacteria during treatment trial with Florfenicol and Enrofloxacin. *World Journal of Fish and Marine Sciences*, 3: 21-36.

## تأثير المكملات الغذائية المستخلصة من تفلّة نبات الجو جوبا على المعاملات الحيوية و البيو كيميائية و معدل مقاومة الأمراض لأسماك البلطي النيلي المصابة بميكروب الأيرومونات هيدروفيلاً

السيد احمد عبد العزيز<sup>١</sup>، أحمد عبده سعيد<sup>١</sup>، عبد العليم فؤاد عبد العليم<sup>١</sup>، سميرة محمد عوض<sup>٢</sup> وإسماعيل عبد المنعم سرحان<sup>٢</sup>

١- قسم الفارماكولوجيا -كلية الطب البيطري- جامعة الزقازيق -مصر

٢- المعمل المركزي لبحوث الثروة السمكية - العباسة-أبو حماد - مصر

### المخلص العربي

فعالية تفلّة نبات الجو جوبا المستخلصة تم تقييمها عن طريق استخدام 240 سمكة من أسماك البلطي النيلي والتي تم تقسيمها الى 8 مجموعات متساوية ( كل مجموعة مكونة من 3 مكررات) وقد تمت التغذية لمدة ٩٠ يوم حيث ان المجموعة الأولى والثانية تتغذى على نظام غذائي متوازن خال من تفلّة الجو جوبا المستخلصة والمجموعة الثالثة والسادسة تتغذى على نظام غذائي متزن مضاف اليه تفلّة نبات الجو جوبا بمقدار ٠,٥ جم/كجم والمجموعة الرابعة والسابعة تتغذى على نظام غذائي متزن مضاف اليه تفلّة نبات الجو جوبا بمقدار ١ جم/كجم وأما المجموعة الخامسة والثامنة فتتغذى على نظام غذائي متزن مضاف اليه تفلّة نبات الجو جوبا بمقدار ٢ جم/كجم حيث تم تغذية اسماك البلطي النيلي على العلائق المختلفة المذكورة سابقا لمدة ٩٠ يوم وقد تم اجراء اصابة للمجموعات الثانية والسادسة والسابعة والثامنة لاسماك البلطي النيلي بميكروب الايرومونات هيدروفيلاً بعد ٧٥ يوم التغذية وتركت المجموعات لمدة ١٤ يوم وتم اخذ عينات دم بعد ٢٤ ساعة من انتهاء التجربة (بعد ٩٠ يوم من التغذية تم اخذ عينات فى اليوم ٩١ بعد وقف التغذية). وقد تم قياس المعاملات الحيوية والكيميائية بالإضافة الى تقييم معدل النجاة ومقاومة الامراض لأسماك البلطي النيلي الحاملة للعدوى. وقد تم اخذ عينات دم من اسماك البلطي النيلي الحاملة للعدوى بعد نهاية التجربة لقياس عدد كرات الدم الحمراء و عدد كرات الدم البيضاء و قياس نسبة الهيموجلوبين وقد تم فصل المصل من عينات الدم، لقياس نسبة البروتين الكلى و الزلال و الجلوبيولين وقد تم تقييم الأثر الوقائي لتفلّة نبات الجو جوبا المستخلصة عن طريق إصابة اسماك البلطي النيلي باستخدام المصل المرضى للميكروب البكتيري ايرومونات هيدروفيلاً و كانت النتائج كالاتى

زيادة عدد كرات الدم الحمراء و عدد كرات الدم البيضاء وزيادة نسبة الهيموجلوبين ونسبة البروتين الكلى و الزلال و الجلوبيولين كما لوحظ نقص فى نسبة معامل الزلال على الجلوبيولين ونقص ايضا فى معدل الوفيات فى المجموعات التي تلقت مستخلص تفلّة نبات الجو جوبا خلال فترة التجربة من النتائج السابقة نستنتج ان إضافة مستخلص تفلّة نبات الجو جوبا الى عليقة اسماك البلطي أدى الى وجود تحسن ملحوظ الحالة الصحية والمناعية الأسماك البلطي النيلي الحاملة للعدوى مما ينتج عنه توصية بإضافة مستخلص تفلّة نبات الجو جوبا الى عليقة اسماك البلطي النيلي