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Phytochemical Constituents of *Ulva Lactuca* and Supplementation to Improve The Nile Tilapia (*Oreochromis Niloticus*) Haemato-Biochemical Status

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Abstract

This study was carried out to investigate the phytochemical constituents and biological screening of green algae ,*Ulva lactuca* methanolic crude extract. The HPLC spectrum profile identified seven phenolic and flavonoid compounds namely: catechin, chlorogenic ,caffeic, quercetin, and major compounds, ellagic acid with 60.87 % and rutin with 33.40% of total area. The GC profile of fatty acids portions represented to relative distribution percentage were 9 compounds, the major fatty acids are lauric acid 55.0 % , caprylic acid 21.2 % , capric Acid was 19.8 and palmitic acid was 1.94% . The 17 amino acids produced from GC spectrum, the percentage of total amino acid were 25.98% .The most abundant amino acids were glutamic acid 2.34% , alanine 2.22 and aspartic 2.09, leucine 1.33 , valine 1.26 , phenylalanine 1.0 , therionine 0.98 and isoleucine 0.9%. of total amino acid.Tilapia (*Oreochromis Niloticus*) Diets were divided into *Ulva* fermentation and multi enzymes, groups each with three different supplementations (LC, PRO, and LC + PRO).The result recorded the diets containing fermented *Ulva* with L-carnitine, probiotic (LC+PRO) was the highest level of red blood cell (RBCs , HGB) by 1.97 cmm , 10.63 g/d . Similarly, increase of Hematocrit (HCt) , WBCs, platelets, and lymphocytes due to the highest level of protein, albumin, and globulin in this diets also, *U. lactuca* contains a number of complex carbohydrates and polysaccharides.This study has revealed that *U. lactuca* seaweed is a rich source of protein (22%) which is nutritionally superior to the terrestrial plant proteins and can be used to provide significant proportions of the protein requirements of fish as well as for human.

Keywords: *Ulva lactuca,* phenolics and flavonids , HPLC, GC, total fatty acid, *Nile tilapia,* hematology and biochemical parameters

Introduction

Ulva lactuca, common seaweed abundantly found along the coasts of Alexandria , Egypt. The genus *Ulva* popularly known as sea-lettuce or green laver is one of the most common and abundant green macroalgae throughout the world [1]. Marine algae are considered as a source of bioactive compounds. Marine macroalgae have been used for healthy feed supplement providing necessary beneficial polysaccharides, fatty acids, amino acids, antioxidants, vitamins and minerals [2]. The phytochemicals from marine algae are extensively used in various industries such as food, confectionary, textile, dairy and paper mostly as gelling, stabilizing and thickening agents [3]. Seaweeds are not a supply of nutrients only but also, contains bioactive compounds such as phenolics, proteins, carbohydrates, antioxidants, minerals, food fibers, vitamins and polyunsaturated fatty acids,[4]. The phytochemical screening of marine algae showed the presence of phenolic compounds. Phyto constit uents such as Phenolic compound, Glycosides, flavanoids, alkaloids, Steroids, terpenoids, sugar, fats …etc [5].

They are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities [6]. So, they could offer an alternative to the demands of other ingredients used in aqua feed. They prefer as food by herbivorous fishes since their stomach have low pH levels and specialize guts required for the digestion of plant, [7].

Moreover, they improve the immune system, antiviral, antimicrobial, improved gut function and stress resistance serves as an alternative for fish meal, [8]. The north coast of Egypt, especially in Alexandria, is rich in sources of macro

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algae. Green macroalgae are commonly found in this coastal area annually [9] . Hematological and biochemical parameters generally provide a good picture of fish health and healthy monitoring [10]. Hematological fish structures are said to be affected by a variety of factors, including species, size, age, body shape, environmental conditions, and dietary ingredients (e.g., source of protein, prebiotic, and vitamins) [11].

Nile tilapia (*Oreochromis niloticus*) is the most important aquaculture species in the world. Egypt is a country where, arguably, the farming of tilapia has its roots [12] . Recently introduced this fish as a targeted species in his aquaculture program. The promotion of this activity will improve inland fisheries production in the country. The challenges facing tilapia production today is to improve feed formulation with natural feed ingredients in order to enhance the fish growth, carcass quality, maximize feed efficiency, minimize fish mortality and reduce production cost.

The aim of the present investigation was firstly to investigate the phytochemical and biological screening of green algae (*Ulva lactuca, UL*) crude extract and polysaccharide and to examine the chemical composition specially bioactive phenolic compounds, amino acid, fatty acid.

Also to evaluate the effect of the use of fermented *Ulva* in combination with L-carnitine and /or probiotic and unfermented *Ulva* in combination with (multienzymes, L-carnitine, and/or probiotic) on hematological and biochemical parameters on the Nile tilapia (*Oreochromis niloticus*).

Materials and Methods

Collection of Algae (*Ulva lactuca*).

Green algae (*U. lactuca*) were collected from Alexandria Coast 2017, which is authenticated by Prof. Dr. Talaat N. Amer, Department of Fish Nutrition Central Laboratory for Aquaculture Research (CLAR), Egypt. The fresh algal biomass was washed with seawater, sun-dried and milled using a laboratory blender 600 g of dried algae were grinded, completely flooded with 1.5 liter of absolute methanol overnight, and filtrated. The filtrates were collected and evaporated using rotary evaporator. The obtained methanolic extract was lyophilized and weighed 80 g. The powder was collected and kept in refrigerator till used.

Extraction of *Ulva lactuca* Phytochemical

Ten grams of *Ulva lactuca* were dissolved in 100 ml of methanol by soaking dried powdered overnight (1:50, w/v) at room temperature. The methanolic extract was then collected by water pump vacuum filtration, condensed by a rotary evaporator (Buchi, Switzerland) at 50°C [13], and lyophilized to obtain powdered crude extract. Each powder was dissolved in 1% saline (NaCl, 0.9 %) which was used for further investigations (tested extract). The phytochemical screening and quantitative estimation of the concentration of chemical constituents were carried.

Determination of *Ulva* Extract By High Performance Liquid Chromatography (HPLC):

i) The analysis of methanol fraction of *Ulva Lactuca* extract was carried out on an : Waters 2690 Alliance HPLC system equipped with a quaternary pump, an on-line degasser, an auto-sampler , and equipped with a Waters 996 photodiode array detector. **ii)** Standard preparation: Mix of eight standards in 20 ml mobile phase then sonicated for 20 min , then filtered using 0.22 µm syringe filter then 10 µl were injected. **iii)** Sample preparation: *U lactuca* methanolic crude extract filtered using 0.22 µm syringe filter then 10 µl were injected. d) HPLC analysis conditions: The separation was carried out on a Zorbax SB-C_{18} column (4.6 mm×250 mm,5 µm)

Mobile phase: Buffer (0.1 % phosphoric acid in water) and Methanol • Mode of elution: using gradient elution : 0–30 min (3%–100% B), There was a 5-min wash with 100% B after each run and equilibrium time was 15 min. Flow rate: 1ml/min • Temperature: Ambient • Wavelength: 254 nm. **iv)** All standards (Catechin ,Chlorogenic acid, Caffeic acid, Rutin, Ellagic acid, Quercetin, Kampeferol) and sample were dissolved in methanol HPLC grade and filtered using 0.22 µm syringe filter then 10 µl were injected.

Gas Chromatography (GC) Analysis:

A Agilent 6890 GC system, fitted with a hydrogen flame ionization detector is used for neutral sugars analysis. The high performance capillary column HP-5 (25 m × 250 µm i.d., 0.25 µm film thickness). The injector (splitless mode) and detector temperatures were 250^0C and 300^oC, respectively. The oven temperature was initially at 120^0C programmed to rise linearly at 4^0C/min until 300^0C .The carrier gas was helium.

Gas Liquid Chromatography (GLC) of Saponifiable Fatty Acids:

The extracted fatty acids of plant and the standards were converted to the corresponding methyl esters using ether solution of diazomethane. The methyl ester of the fatty acids were analyzed with GC apparatus. The fraction of fatty acids methyl ester was conducted using (GLC) column.

Peak identification was performed by comparing the relative retention time of each compound with those of standard materials. The relative proportions of each individual compound were estimated as the ratio of the partial area to the total area .

Gas Liquid Chromatography (GLC) of Amino Acids:

In order to determine amino acid profiles, the dried algae samples were subjected to hydrolysis according to Blackburn (1978) and Walker (1996). Amino acid analysis was performed using using (GLC) column of amino acid analyzer. The amino acid profile values are expressed in percentage of total amino acid content.

Diets Formulations and Preparation:

In the present study , a multi-activity feed enzyme (Natuzyme) used which produced by Bioproton Pty Ltd., Sunnybank, Queensland, Australia , composed of (cellulase, xylanase, ß-glucanase, protease, α- amylase, phytase and pectinase)

The probiotic used in this study was a commercial formulation of dried probiotic bacteria (*Lactobacillus* sp., produced by Plexo pharmaceutical Industry, Cairo, Egypt) containing *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus delbrueckii, Bacillus subtilis, Saccharomyces cerevisiae,* and *Rhodopseudomonas palustris*.

The L-carnitine was prepared from the MEPACO Arab Co. for Pharma and Medical Plant, Nasr City, Cairo, Egypt.

Preparation and Fermentation Procedure of Seaweeds:

The dried seaweeds were ground well in the laboratory, pulverized sieved through a 0.3 mm mesh and used as raw seaweed powder and raw material for fermentation.

Microbial fermentation of the seaweed was carried out in the fermenter vessel. The dried seaweed powder to seawater in the ratio of 1:9 (seaweed: seawater) was taken in the fermenter vessel. Each 10 ml of *Lactobacillus* spp. and *Saccharomyces cerevisiae* was inoculated at a concentration of 1.15 x 104 and 2.45 x 104 cfu/ml, respectively.

The sugar substrate , dextrose was added at the rate of 5%w/v of base material. The fermentation was carried out until the pH reached at 4.00. the pH between 4 and 5 is desired for fermentation of feed ingredients because when the pH is below 4.00, the feed intake decreases and above 5.00, microbial spoilage is likely to occur [15].

The fermented seaweed silage was collected from the fermenter and dried in a hot air oven at 60°C for 2 days. The fermented seaweed powder is then used for feed preparation.

The experiment was conducted to compare two different methods:

i) *Ulva* fermentation (FER) and adding multi enzymes, 1.5 g/kg (MEM) to plant *Ulva* based diets, each with three trials of [350 mg/kg L-Carnitine (LC), 0.3% probiotic (PRO) and LC + PRO] using (2x3) factorial design.

ii) Six isonitrogenous and isocaloric diets were formulated with natural ingredients to provide 28% protein and 425 kcal/100 g diet according to the known nutritional requirements of tilapia National Research Council [16].

Diets were divided into *Ulva* fermentation and multi enzymes, groups each with three different supplementations (LC, PRO, and LC + PRO).

Fish and Husbandry Conditions:

Nile tilapia (*Oreochromis niloticus*) fry were obtained from fish Hatchery at the Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abo-Hammad, Sharqia, Egypt. Fish were held in an indoor tank and fed the basal diet (T_1) for two weeks as an acclimation period to the laboratory conditions prior to the trial. Twenty fish with an average initial body weight of (5.14±0.08 g) were weighed and stocked into each 100L glass aquaria (3 replicates of 6 treatments). Half of the water in each aquarium was changed daily to avoid accumulation of the metabolites. Each aquarium was supplied with an air stone for continuous aeration using an electrical air pump to maintain oxygen level. All fish were fed to apparent satiation, twice a day, 6 days/week for 12 weeks. Fish in each aquarium were sampled biweekly. Dead fish were daily recorded and removed. At the end of the study, fish were individually weighed. **(Table 1)**.

Table 1. Composition and Proximate Chemical Analyses (% On Dry Matter Bases) Of the Experimental Diets.

Ingredients	Ferm.			MEM		
	LC	Pro	LC + Pro	LC	Pro	LC + Pro
Soybean meal	51	51	51	51	51	51
Ulva	15	15	15	15	15	15
Wheat bran	17	17	17	17	17	17
Y.corn	7	7	7	7	7	7
Wheat flour	2	2	2	2	2	2
Fish oil	3	3	3	3	3	3
Starch	1.965	1.7	1.665	1.815	1.55	1.515
Vitamins premix[1]	1	1	1	1	1	1
Minerals premix[2]	2	2	2	2	2	2
LC	0.035	0	0.035	0.035	0	0.035
Prebiotic	0	0.3	0.3	0	0.3	0.3
MEM	0	0	0	0.15	0.15	0.15
Total	100	100	100	100	100	100
Diets Comp. :						
Moist. %	8.98	8.76	8.54	8.56	8.55	8.9
C.P. %	27.28	27.16	27.18	27.16	27.16	27.17
Lipid %	8.23	8.07	8.21	8.74	8.35	8.38
Fiber %	7.25	7.43	7.43	7.12	7.42	7.38
Ash %	11.33	11.32	11.14	11.12	11.12	11.25
NFE %	45.91	46.02	46.04	45.86	45.95	45.82
Gross Energy	420.13	418.85	420.36	424.52	421.2	421.02

1-Vitamin premix (per kg of premix): thiamine, 2.5 g; riboflavin, 2.5 g; pyridoxine, 2.0 g; inositol, 100.0 g; biotin, 0.3 g; pantothenic acid, 100.0 g; folic acid, 0.75 g; para-aminobenzoic acid, 2.5 g; choline, 200.0 g; nicotinic acid, 10.0 g; cyanocobalamine,0.005 g; a-tocopherol acetate, 20.1g;menadione,2.0g;retinol palmitate,100,000 IU; cholecalciferol, 500,000 IU. **2-** Mineral premix (g/kg of premix): $CaHPO_4.2H_2O$, 727.2; $MgCO_4.7H_2O$,127.5; KCl 50.0; NaCl, 60.0; $FeC_6H_5O_7.3H_2O$, 25.0; $ZnCO_3$, 5.5; $MnCl_2.4H_2O$, 2.5; Cu (OAc)2. H_2O, 0.785; $CoCl_3.6H_2O$, 0.477; $CaIO_3.6H_2O$, 0.295; $CrCl_3.6H_2O$, 0.128; $AlCl_3.6H_2O$, 0.54; Na_2SeO_3, 0.03. **3** -Nitrogen-Free Extract (calculated by difference) = 100 – (protein + lipid + ash + fiber).**4-** Gross energy (GE) was calculated from NRC, (1993) as 5.65, 9.45, and 4.11kcal/g for protein, lipid, and carbohydrates, respectively.

Physiological Measurements:

At the end of the feeding trial, three fish from each aquarium were taken for physiological investigation. Fish were anaesthetized using buffered tricaine methanesulfonate (20 mg/L), and blood was collected from the caudal vein with a sterile syringe and divided equally among three clean and dry tubes. The first part was centrifuged at 3,000 g for 15 min and the serum was stored at −20◦C for further assays. The second part was mixed with sodium fluoride as an anticoagulant and centrifuged at 3000 g for 15 min for separation of plasma for glucose analysis. The last part was mixed with EDTA solution for measuring hemoglobin (Hb), red blood cell (RBCS), and hematocrit (HCt). Hemoglobin level was determined calorimetrically using a spectrophotometer . Hematocrit was determined using the microhaematocrit method [17] . Red blood cells were determined according to the method described by [18] .Total protein content was determined calorimetrically according to [19] .Colorimetric determination of serum albumin was performed according to [20] using a spectrophotometer. Cholesterol is estimated as a colored complex according to the method of [21] . Creatinine was determined calorimetrically according to [22]. Glucose was determined calorimetrically according to [23] . Activities of aspartate

Physiological Measurements:

aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically according to [24]

Statistical analysis:

Data were submitted to two-way ANOVA and were expressed as the mean ± SD of the replicates. Differences were considered significant if P was less than 0.05 [25]. All statistical analyses were using SAS, [26] . Significant differences ($p \leq 0.05$) among means were tested by the method of Duncan [27].

Results and Discussion

Phenolic and Flavonoid Composition of *Ulva lactuca*

Further screenings of the phenolic compounds were achieved through HPLC analysis. Phenolic content of the methanolic extract of *Ulva lacuca* are summarized in **(Table 2 and Figure 2).** Major Phenolic content, were ellagic acid at R_t at 19.876 with 60.87 % of area followed by Rutin at R_t at 19.467 with 33.40 % and kampeferol R_t at 24.366 & other phenolics with low concentration (catechin , chlorogenic acid, caffeic acid and quercetin), **(Figure 1).** HPLC analysis revealed the presence of phenolic active constituents in high content acting to increase its bioactivity

Table 2: Phenolics and Flavonids Compounds Identified in *Ulva Lactuca* HPLC.

No	Compounds	R_t	% Area	Height
1.	Catechin	15.25	1.1	6792
2.	Chlorogenic acid	15.63	2.07	15191
3.	Caffeic acid	16.570	0.59	3148
4.	Rutin	19.467	33.40	191947
5.	Ellagic acid	19.876	60.87	382703
6.	Quercetin	23.770	0.68	5142
7.	Kampeferol	24.366	1.1	8191

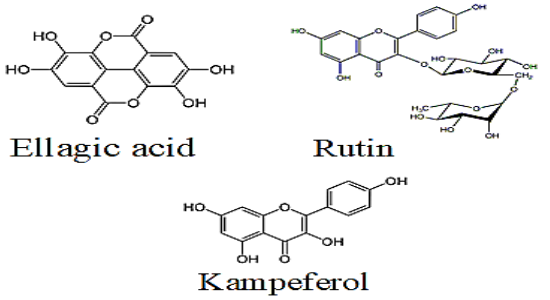
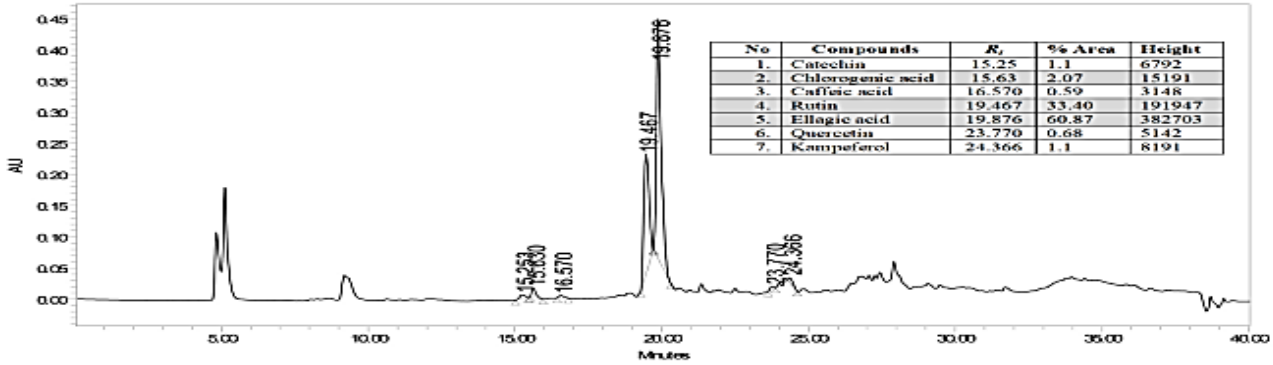


Figure 1 : Chemical structures of major favonoids the methanolic extract of *Ulva lacuca* which have been identified by HPLC



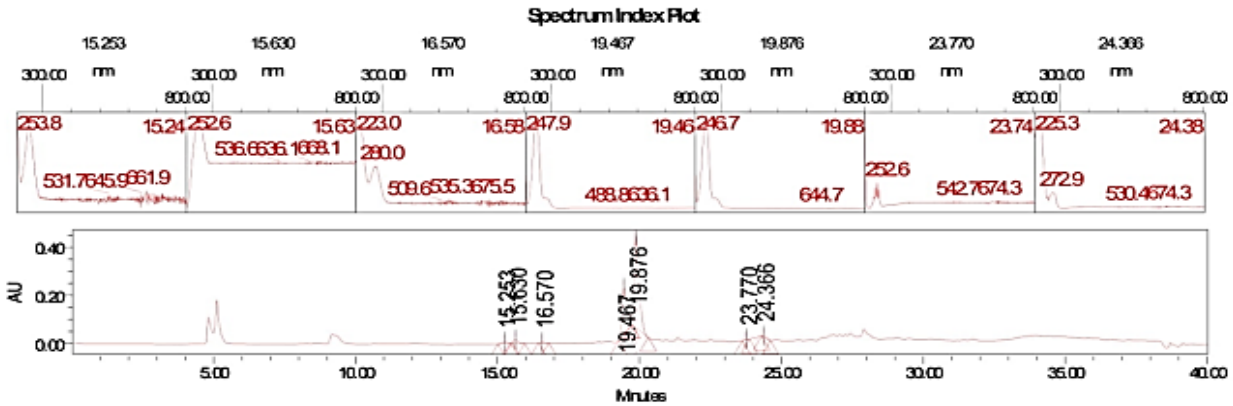


Figure 2. HPLC Chromatogram of phenolic acids and flavonoids. catechin, chlorogenic ,caffeic, rutin, ellagic acid, quercetin and kampeferol according to the standered

Fatty acid composition of *Ulva lactuca*

The fatty acids compositions of investigated *U. lactuca* macroalgae are listed in **(Table 3)** . The saturated and polyunsaturated portions represented to relative distribution percentage was 9 compounds, the major fatty acids are lauric acid 55.0 % , caprylic acid 21.2 % , capric acid was 19.8 and palmitic acid was 1.94%.

In our study showed that *U. lactuca* seaweed has higher total levels of fatty acids monounsaturated than polyunsaturated fatty acids . Also this seaweed contained the essential fatty acids C18:2 (linoleic acid) and C18:3 (linolenic acid),

Amino Acids Composition of *Ulva lactuca*

In the following GC experiment, *Ulva lactuca* contain 17 amino acids which included both free and combined amino acids, Total amino acid percentage was 25.98% . The most abundant amino acids were glutamic acid 2.34 , alanine 2.22 and aspartic 2.09 , leucine 1.33%, valine 1.26 , phenylalanine 1.0 % , therionine 0.98 & isoleucine 0.9%. (**Table 4, Fig.3).**

Table 3: Fatty Acid Composition of Macroalgae *U. Lactuca* (g/100 g of Total Fatty Acid).

NO	Fatty Acids		Relative distribution %
1.	C 8:0	Caprylic acid.	21.2
2.	C10:0	Capric Acid	19.8
3.	C12:0	Lauric Acid	55.0
4.	C14:0	Myristic Acid	0.13
5.	C16:0	Palmitic Acid	1.94
6.	C16:1	Palmitoleic	0.25
7.	C18:0	Stearic	0.23
8.	C18:1	Oleic	0.78
9.	C18:2	Linoleic	0.54

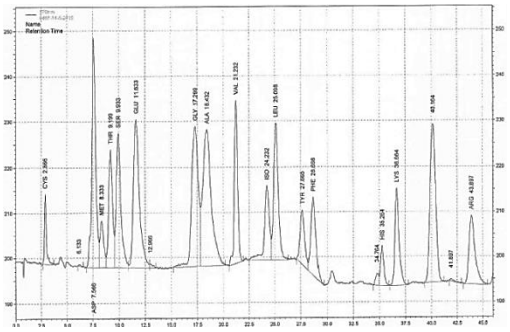


Figure 4 : GC spectrum of **Amino Acids Composition of *Ulva lactuca***

Table 4: Amino Acids Composition of *Ulva lactuca*

	Amino Acids		%
1.	Aspartic	(ASP)	2.09
2.	Threonine	(THR)	0.98
3.	Serine	(SER)	0.96
4.	Glutamic acid	(GLU)	2.34
5.	Glycine	(GLY)	1.20
6.	Alanine	(ALA)	2.22
7.	Valine	(VAL)	1.26
8.	Isoleucine	(ILE)	0.90
9.	Leucine	(LEU)	1.33
10.	Tyrosine	(TYR)	0.68
11.	Phenylalanine	(PHE)	1.00
12.	Histidine	(HIS)	0.31
13.	Lysine	(LYS)	0.89
14.	Arginine	(ARG)	1.17
15.	Proline	(PRO)	0.79
16.	Cysteine	(CYS)	0.31
17.	Methionine	(MET)	0.44

Bio-Chemical Changes:

Fish fed the third diet, containing fermented *Ulva* and L-carnitine, probiotic (LC+PRO) where the sixth diet containing normal *Ulva* in combination with multienzyme mixture, L-carnitine, probiotic, (LC+ PRO) . The result recorded the highest of red blood cell (RBCs , HGB) at the third diet (1.97 cmm , 10.63 g/d) and the sixth diet (1.96 cmm , 10.73 g/dl). Similarly, The volume percentage (vol%) of red blood cells (RBC) in blood value Hematocrit (HCt) of third diet, was 25.10 and for sixth diet was 19.43 % also increase in WBCs, platelets, and lymphocytes was found in fish fed the third and sixth diets (**Table 5) .** The results concluded that the third and sixth diets exhibited the highest total protein, albumin, and globulin in comparison with experimental diets.

Haemato- Biochemical Changes:

The results revealed that fish that consumed the third diet, containing fermented *Ulva* and L-carnitine, probiotic and the sixth diet containing normal *Ulva* in combination with multi enzyme mixture, L-carnitine, probiotic, recorded the highest values of Hb and RBCs. Similarly, a significant increase in WBCs, platelets, and lymphocytes was found in fish fed the third and sixth diets. Red blood cells (RBCs) and HCt were not significantly different among various experimental treatments. Fish fed the third diet (containing fermented *Ulva*, L-carnitine, probiotic) and the sixth diet (containing normal *Ulva* in combination with multienzyme mixture, L-carnitine, probiotic) recorded the highest value of Hb and RBCs (10.63 g/dl, 1.97 cmm) and (10.73 g/dl, 1.96 cmm), respectively **(Table 6).**

Table 5 . Change in *Nile tilapia* Hematological Parameters.

Treat.		Blood Parameters (hematology)						
		RBCs	HGB	HCt	MCV	PLT	WBCs	LYM
Ferm.	LC	1.63±0.04a	9.40±1.01b	21.17±2.75a	126.67±2.73b	43.33±8.11ab	49.64±1.64b	30.70±1.41b
	PRO	1.67±0.21a	9.13±0.20b	19.84±1.55a	121.33±7.36b	46.00±13.80a	64.59±10.18a	49.63±9.40a
	LC+PRO	1.97±0.18a	10.63±0.89a	25.10±2.92a	127.33±7.31b	48.33±9.91a	65.98±7.97a	49.84±10.61a
MEM	LC	1.81±0.10a	10.10±0.64ab	23.46±1.69a	119.67±9.94b	40.33±10.17b	51.16±4.93b	31.61±1.27b
	PRO	1.80±0.11a	9.37±0.67b	24.40±1.84a	116.67±6.44b	37.33±8.57b	63.86±11.58a	48.23±10.83a
	LC+PRO	1.96±0.03a	10.73±0.15a	19.43±2.45a	135.00±6.24a	47.33±23.14a	63.53±9.98a	50.11±8.12a
Pooled Means:								
Ferm.		1.76±0.10g	9.72±0.45g	22.04±1.47g	125.11±3.24g	45.89±5.48g	60.07±4.58g	43.39±5.19g
MEM		1.86±0.05g	10.07±0.34g	22.43±1.27g	123.78±4.80g	41.67±7.85g	59.52±5.09g	43.31±4.90g
LC		1.72±0.06x	9.75±0.56xy	22.31±1.53x	123.17±4.87y	41.83±5.86x	50.40±2.35y	31.15±0.87y
PRO		1.74±0.11x	9.25±0.32y	22.12±1.48x	119.00±4.40y	41.67±7.52x	64.23±6.90x	48.93±6.42x
LC+PRO		1.97±0.08x	10.68±0.40x	22.27±2.13x	131.17±4.63x	47.83±11.26x	64.76±5.74x	49.98±5.97x

Means having the same letter in the same column is not significantly different (P<0.05).Red blood cells (RBCs), Hemoglobin (HGB) , Hematocrit (HCt), Mean corpuscular volume (MCV), Platelets (PLT), White blood cells (WBCs) , Lymphocytes (LYM)

Table 6 : The Bio-chemical Changes in *Nile tilapia*

Treat.		Biochemical Parameters						
		Total Protein (g/dl)	Albumine (g/dl)	Glucose (mg/ dl)	Cholesterol (mg/ dl)	Creatinine (mg/ dl)	ALT (GPT) U/L	AST (GOT) U/L
Ferm.	LC	2.44±0.27a	0.65±0.05a	49.67±17.16b	70.67±14.29b	0.17±0.02a	7.03±0.29b	13.60±1.08a
	PRO	2.22±0.60b	0.63±0.02a	51.67±2.08b	73.33±4.93b	0.14±0.03a	10.37±0.35a	14.13±2.35a
	LC+PRO	2.60±0.28a	0.73±0.06a	60.67±23.09a	85.00±8.72a	0.14±0.04a	9.60±2.00ab	13.33±1.86a
MEM	LC	2.38±0.34a	0.71±0.12a	50.00±1.73b	80.67±14.01ab	0.13±0.03a	7.77±0.40b	13.53±1.29a
	PRO	2.22±0.08b	0.70±0.03a	45.67±4.62b	80.67±5.03ab	0.15±0.04a	8.63±1.56ab	13.40±1.55a
	LC+PRO	2.69±0.31a	0.74±0.08a	50.00±1.73b	82.67±4.73a	0.17±0.02a	8.83±0.75ab	13.83±2.75a
Pooled Means:								
Ferm.		2.42±0.13a	0.67±0.02a	54.00±5.10a	76.33±3.65a	0.15±0.01a	9.00±0.61a	13.69±0.54a
MEM		2.43±0.10a	0.72±0.03a	48.56±1.13a	81.33±2.62a	0.15±0.01a	8.41±0.34a	13.59±0.57a
LC		2.41±0.11xy	0.68±0.04x	49.83±4.45x	75.67±5.63x	0.15±0.01x	7.40±0.21y	13.57±0.43x
PRO		2.22±0.16y	0.66±0.02x	48.67±1.87x	77.00±2.45x	0.15±0.01x	9.50±0.57x	13.77±0.74x
LC+PRO		2.65±0.11x	0.74±0.03x	55.33±6.44x	83.83±2.61x	0.16±0.01x	9.22±0.58x	13.58±0.87x

This is in line with (28), who found that treatments received a mixture of *Saccharomyces cerevisiae* and exogenous digestive enzymes revealed higher level of red blood cell counts, hematocrit, and hemoglobin. Also of [29] , who observed a significant increase in the number of RBCs, WBCs, Hb concentrations, and PCV% in Nile tilapia receiving two types of probiotics (*Bacillus subtilis* and *saccharomyces cerevisiae*). The hepato-stimulatory and hepato-protective effect of probiotics leads to improvement of the erythrogram parameters [30]. In relation to the fermentation effect, [31] cited that Hb, HCt, RBCs, and WBCs were not significantly different in fish fed different

sunflower meal supplementation applied to the solid-state fermentation process with *Saccharomyces cerevisiae* (YFSFM) or *Bacillus subtilis* (BFSFM).

The concentration of total protein in blood plasma is used as a basic index for the health status of brood fish [32] as serum or albumin concentrations have diagnostic value in laboratory animals as it relates to normal dietary status and the integrity of the vascular and liver function. The results concluded that the third containing fermented *Ulva* and L-carnitine, probiotic and sixth diets containing normal *Ulva* in combination with multienzyme mixture, L-carnitine, probiotic, exhibited the highest total protein, albumin, and globulin in comparison with experimental diets. This match with those of [33] who showed that protease enzyme addition increased total protein levels, globulin, and albumin in the serum of Nile tilapia fed diets with high incorporation levels of cotton seed meal and supplemented with exogenous protease. Similarly, [34] used three types of probiotics, multi-strain probiotic (6 × 107 cfu / g), *Bacillus subtilis* (1 × 1011 cfu / g), and *Saccharomyces cerevisiae* (2.6 × 1010 cfu / g) in Nile tilapia diet and showed an increase in total protein and globulin in fish fed different probiotic species.

The measurement of AST and ALT is indicative of general systemic nutritional status and the integrity of the vascular system and liver function [35]. Increased activities of serum AST and ALT in fish may be attributed to increased synthesis of enzymes by the liver and/or possible leakage of enzymes across damaged plasma membranes [36] **.** The result of the following study revealed that serum AST levels of different treatments were not significantly different, while the lowest serum AST level recorded in the third diet. Serum ALT levels decreased significantly with the first diet. Consistent with our results, [34] used three types of probiotics, multi-strain probiotics (6 × 107 cfu/g), *Saccharomyces cerevisiae* (2.6 × 1010 cfu/g) and *Bacillus subtilis* (1 × 1011 cfu/g) and in the diet of Nile tilapia, and showed that ALT and AST levels were reduced in fish fed with different probiotic species. In addition [37] reported the addition of a Nutrasexylam © enzyme supplement containing a mixture of α-amylase (40,000 /g) and β-xylanase (6,300u/g) had no adverse effect on fish health and that the concentrations of AST and -ALT in plasma was normal. (38) Concluded that higher dietary intake of a fermented soybean meal diet caused elevation of ALT and AST levels in Nile tilapia. These findings are supported by [31], who showed that fish fed with different concentrations of sunflower meal applied to solid-state fermentation process with *Bacillus subtilis* (BFSFM) or Saccharomyces cerevisiae (YFSFM) revealed greater serum ALT and AST activity.

Conclusion

This study has revealed that *U. lactuca* seaweed is a rich source of many important nutrients. *Ulva* sp. could be considered as a source of high levels of protein (22%) which is nutritionally superior to the terrestrial plant proteins and can be used to provide significant proportions of the protein requirements of fish as well as for human. It contains a number of complex carbohydrates and polysaccharides. It constitutes a good alternative source of essential amino acids and of some polyunsaturated fatty acids. The marine organisms that have many phytochemical constituents and show many biological activities and on the other hand safe and not toxic such as *Ulva lactuca*.

Using fermented Ulva and inclusion of exogenouss digestive enzymes in combination with L-carnitine and/or probiotic is capable of improving hematological and biochemical parameters of Nile tilapia.

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