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Evaluation of *Ulva lactuca* fermentation and exogenous multi-enzymes supplementation in combination with L-carnitine and probiotic on optimizing plant-based diets utilization for the Nile tilapia (*Oreochromis niloticus*).

Talaat N. Amer [1], Amany M. El-mousalamy [2] , Sally Z. mohamed [1]* and Yossef M. Ali [3]

1. Fish Nutrition Department,Central Laboratory for Aquaculture Research Center, Agriculture Research Center, Egypt.
2. Chemistry Department, Faculty of science, Zagazig University, Egypt.
3. Biochemistry Department, Faculty of Veterinary Medicine, Zagazig University, Egypt

*Corresponding Author: sallyzidan100@yahoo.com

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ABSTRACT

A (2X3) factorial design was conducted to evaluate using fermented *Ulva lactuca* (FER) and exogenous multi-enzymes, Natuzyme® (MEM, 1.5 g/kg) supplementation in combination with L-carnitine (LC, 350 mg/kg) and/or probiotic (PRO, 0.3%/kg) on growth performance and feed utilization of Nile tilapia (5.14±0.08 g initial body weight) fed plant-*Ulva* based diets over a 12-weeks feeding trial. Six isonitrogenous and isocaloric diets were formulated to provide 28% protein and 425 kcal/100 g diets which divided into FER and MEM groups each with three different supplementations (LC, PRO and LC + PRO). Results of the present study revealed that fish fed on the third diet (FER + LC + PRO) and last diet (MEM + LC + PRO) were significantly higher in average body weight, weight gain, and relative body weight gain than other treatments without significant differences between the two treatments. Fish fed (FER + LC + PRO) diet and (MEM + LC + PRO) diet consumed more feed than other treatments. No significant differences were detected among different fish groups for whole-body composition. The results of the present study suggested that using fermented Ulva lactuca and inclusion of exogenous multi-enzymes in combination with L-Carnitine and/or probiotics have beneficial effects on improving growth performance, feed utilization, and reducing feed cost of Nile tilapia.

INTRODUCTION

Nile tilapia, *Oreochromis niloticus* are considered as the most common and popular fish in Egypt. Egypt is a country where, arguably, the farming of tilapia has its roots **(Stickney, 2006)**. Tilapia aquaculture is rapidly expanding with a global production of about 3.95 million metric tons in 2011 and estimated to increase to 9.2 million metric tons by the year 2030 **(FAO, 2014)**.

Fishmeal is generally considered to be the most ideal protein source for aquatic animals, despite its static global production, seasonal/geographical variability in quality and composition, and concern as a vector of contamination **(Trushenski *et al.*, 2006)**. Over the past decades, there have been rapid increases in global aquaculture and demand, as well as price and competition for this valuable feedstuff. Researchers have been studying to replace animal protein sources with proteins derived from plant materials (e.g. soybean meal, cereal by-products and legumes) or some feed additives for stimulate to the growth in order to reduce the dependence for fishmeal which may provide more economic and environmentally friendly aquaculture **(Li *et al.*, 2010; Amer, 2012)**.

Ulva species is a good source of protein, pigments, minerals and vitamins, and is especially rich in vitamin C **(Ortiz *et al.*, 2006)** Vitamin C promotes lipid metabolism, which may result in the alteration of body composition and nutrient deposition in fish, and thus may reduce carcass lipid and increase protein levels **(Ji *et al.* 2003)**. In recent years, Ulva species have become important macro algae, which have been investigated as a dietary ingredient for a wide range of fish species. However the presence of high crude fiber and low protein content are issues for low inclusion of seaweeds in aqua feeds.

Fermentation is a simple and cheap method which might considerably decrease crude fiber content and increase protein value. Fermentation will help feed manufacturers to replace fishmeal to certain levels and help in reducing the feed cost and thereby increasing the profitability of aquaculture systems **(Felix and Brindo, 2008)**. Fermentation can be performed on any kind of seaweeds including rhodophyta, chlorophyta and phaeophyta and fermentation efficiency is relatively high in chlorophyta because of the high content of glucose availability **(Uchida and Murata, 2002)**.

Also, growth response and feed utilization were improved with enzyme supplementation, suggesting that the negative effects of plant ingredients were compensated to some extent by addition of the enzymes. Supplementation with enzymes is effective to eliminate the anti-nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved fish performance **(Lin et al. 2007; Soltan 2009)**.

L-carnitine (l-h-hydroxy-g-N, N, N-trimethylaminobutyric acid) is a nonessential organic nutrient that is required for the entry of long-chain fatty acids into mitochondria for b oxidation. L-carnitine serves as a growth promoter, helping in the utilization of high fat levels in the diet and thus providing protein sparing effect. Numerous studies have been conducted to assess the roles of L-carnitine on enhancing animal production and improving body composition for human consumption **(Harpaz, 2005)**.

Use of probiotics, that beneficially affect the host by selectively stimulating the growth and/or activating the metabolism of health-promoting bacteria in the intestinal tract, is a novel concept in aquaculture **(Gibson *et al.*, 2004).** One possible means of accomplishing this is to introduce supplements of probiotics into the diet **(Reza *et al.*, 2009)**. The use of probiotics offers a promising alternative to the use of antimicrobial

chemicals in aquatic animals. Probiotics are defined as beneficial microorganisms that when supplied in appropriate amounts, confer positive effects on the health of the host. They have multiple benefits for the host, including providing nutrients and enzymatic digestion that improves metabolism and enhances growth, stimulating beneficial microflora in the GI tract, competing for adhesion sites with harmful bacteria to inhibit the growth of pathogenic microorganisms and enhancing the host's innate immunity against pathogen infection **(Akhter *et al*., 2015)** and **(Hai , 2015).**

The objective of this study was to evaluate using fermented *Ulva lactuca* and exogenous multi-enzymes supplementation in combination with L-carnitine and/or probiotic on growth performance and feed utilization of Nile tilapia fed on all plant-based diets.

MATERIALS AND METHODS

Diets formulation and preparation:

The seaweeds *Ulva lactuca* were collected from Alexandria beach, washed well in fresh water to eliminate salts and all algae and outruns on it. They were dried at a temperature (60 – 70°C) to avoid release of nutrients important to marine fish larvae. The dried seaweeds was ground in grinder mixer and stored in plastic sacs until adding to the diets.

Natuzyme® used in the present work is a powdered micro-granulated multi-enzyme preparation commercially available feed additive produced by Bioproton Pty Ltd., Sunnybank, Queensland, Australia (Table 1).

The probiotic used in this study was a commercial formulation of dried probiotic bacteria (*Lactobacillus* sp., producted by Plexo pharmaceutical industry, Cairo, Egypt) containing *Lactobacillus plantarum, Lactobacillus fermentum, Lactobacillus delbrueckii, Bacillus subtilis, Saccharomyces cerevisiae, Rhodopseudomonas palustris*. The L-carnitine was prepared from the MEPACO Arab Co. For Pharma & Medical Plant, Nasr City, Cairo, Egypt.

Preparation and fermentation procedure of seaweeds:

Seaweeds (*Ulva sp*.) were collected from Alexandria city coast (attached to rocks) and prepared as described by **Amer and El-Tawil (2011)**. Seaweeds were rinsed with fresh water then distributed on plastic sheet and left to dry in the sun, then put in oven drying at (60 -70 °C) to be dried. After that, it was crushed and grinded through a food grinder mixer and kept dry until it used in diets formulation.

The dried seaweeds were grounded well in laboratory pulverizer 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 cfu/ml 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 till the pH reached at 4.00. A 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 **(Lee *et al.*, 2004)**. The fermented seaweed silage was collected from the fermenter and dried in a hot air oven at 60 0C for 2 days. The fermented seaweed powder is then used for feed preparation.

The experiment was conducted to compare two different methods: *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) or LC+PRO] using (2X3) factorial design.

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 **(NRC, 2011)** (Tables 2 and 3). Diets were divided to FER and MEM groups each with three different supplementations (LC, PRO and LC + PRO).

Table 1. Composition of the exogenous multi-enzyme complex (Natuzyme®) used in this experiment.

Enzyme	Activity* (unit/g) at 30°C, pH 7.2
Cellulase	5000
Xylanase	10000
ß-glucanase	1000
Protease	6000
***α*- amylase**	1800
Phytase	500
Pectinase	140

*Activity (unit/kg): the amount of the enzyme that catalyses the conversion of 1 µM of substrate per minute under specified conditions (temperature and pH).

The dietary ingredients were homogeneously grounded to 500 µm and thoroughly mixed. Then sufficient amount of water (about 400 ml/kg diet) was added and mixed to obtain stiff dough which was passed through a 1.5 mm die mincer. The pelleted diets were air dried by electric fan at room temperature for 24 hrs. All diets were packed in sealed plastic bags and kept stored at 4 °C until use.

Table (2): Ingredients and chemical analysis of experimental diets (on dry matter basis)

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
Prebiotec	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, 20.1 g; menadione, 2.0 g; retinol palmitate, 100,000 IU; cholecalciferol, 500,000 IU.

2- Mineral premix (g/kg of premix): CaHPO4.2H2O, 727.2; MgCO4.7H2O, 127.5; KCl 50.0; NaCl, 60.0; FeC6H5O7.3H2O, 25.0; ZnCO3, 5.5; MnCl2.4H2O, 2.5; Cu(OAc)2.H2O, 0.785; CoCl3.6H2O, 0.477; CaIO3.6H2O, 0.295; CrCl3.6H2O, 0.128; AlCl3.6H2O, 0.54; Na2SeO3, 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.

Table (3): Proximate chemical analysis of *U. lactuca* (% on dry matter basis) and other dietary ingredients.

Item	*U. lactuca*	HFM	SBM	WB	CNM
Dry matter	15.84	92.42	93.8	91.01	90.37
Crude protein	22.46	72.21	44.03	14.98	9.55
Total lipids	15.75	11.42	1.31	4.41	3.98
Ash	16.94	11.14	5.95	3.34	1.5
Crude fiber	0.78	0.55	5.62	10.84	5.14
NFE	44.07	4.68	43.09	66.43	79.83
GE	456.40	480.21	372.76	347.1	362.98

-Herring fish meal (HFM), soybean meal (SBM), wheat bran (WB), and corn meal (CNM).

Fish and husbandry conditions:

The experiment was performed at the department of fish nutrition, Central Laboratory for Aquaculture Research (CLAR) Abbassa, Abu-Hammad, Sharkiya governorate, Egypt. Nile tilapia (*Oreochromis niloticus*) fry were obtained from CLAR hatchery ponds. 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 of 100 L glass aquaria (3 replicates of 6 treatments). One half of 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. During the course of the experiment, all fish were collected from each aquarium every two weeks and collectively weighed.

Sampling, Analytical Procedure and Measurements:

Fish were sampled at the beginning and at the end of the trial from each tank, dried and immediately stored at −20 °C pending analyses. Diets and carcass samples were submitted to proximate composition analysis according to the standard methods of AOAC (1990) for moisture, crude protein, total lipids, and ash. Moisture content was estimated by drying the samples at 85°C in a drying oven (GCA, model 18EM, Precision Scientific group, Chicago, Illinois, USA) until a fixed weight was achieved. Crude protein was estimated by multiplying the nitrogen content which was determined using a micro-Kjeldahl apparatus (Labconco Corporation, Kansas, Missouri,USA) by 6.25. Lipid content was determined by petroleum ether extraction in a Soxhlet apparatus (Lab-Line Instruments, Inc., Melrose Park, Illinois,USA) at 40-60 °C for 16 h. Ash was determined by combusting dry samples in a muffle furnace (Thermolyne Corporation, Dubuque,

Iowa, USA) at 550 °C for 6 h. Crude fiber was estimated according to Goering and Van Soest (1970) and the nitrogen free extract (NFE) was calculated as:

NFE (%) =100 − (% crude protein + % crude lipid + % crude fiber + % ash). Gross energy was calculated according to NRC (1993).

Growth performance parameters:

The following equations were used for the weight gain, daily weight gain, specific growth rate and survival rate, respectively.

Weight gain (WG) = W_1-W_0.

Daily weight gain (DWG) = (W_1-W_0)/T.

Specific growth rate (SGR%/day) = [(Ln W_1− Ln W_0)/T] × 100.

Where, Ln = natural log, W_0 = Initial body weight (g), W_1= Final body weight (g) and T= Time (day).

Survival rate (%) = 100 × (fish No. at the end/ fish No. stocked at the beginning).

Feed utilization parameters:

The following equations were used for the feed intake, feed conversion ratio**,** protein efficiency ratio, protein productive value and energy retention, respectively.

Feed intake (FI) = total feed consumed over experimental period (g)/ number of fish.

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

Protein efficiency ratio (PER) = total weight gain (g)/protein intake (g).

Protein productive value (PPV %) = 100 (protein gain/protein intake).

Energy retention (ER %) = 100 (gross energy gain/gross energy intake).

Water quality analysis:

Water samples were collected biweekly throughout the experimental period from each aquarium. Water temperature and dissolved oxygen were measured in each aquarium with an YSI model 58 oxygen meter (Yellow Spring Instrument Co., Yellow Spring, Ohio, USA). While the pH was measured using a pH–meter (Digital Mini-pH Meter, model 55, Fisher Scientific, USA). Unionized ammonia, total alkalinity and total hardness were determined according to **Boyd and Tucker (1992)**.

Statistical Analyses:

Data were submitted to one-way ANOVA and were expressed as the mean ± SD of the replicates. Differences were considered significant if P was less than 0.05. All statistical analyses were using SAS, **(SAS Inc., 2002)**. Significant differences ($p \leq 0.05$) among means were tested by the method of **Duncan (1955)**.

Economical evaluation:

A simple economic analysis was conducted for different experimental treatments to estimate the cost of feed required to produce a unit of fish biomass. The estimation was based on local retail sale market price of all the dietary ingredients at the time of the study. These prices were as follows: herring fish meal, 17.00; soybean meal, 4.00; yellow corn meal, 2.50; wheat bran, 2.25; fish oil 30; starch 6.00; vitamins mixture, 10; minerals mixture, 4.50; probiotic, 150 LE/Kg; L-Carnetine, 440 LE/kg and multi-enzymes mixtures, 120 LE/kg.

RESULTS

Water temperature ranged from 27.00 to 27.5°C, while pH ranged from 7.3 to 7.6 Dissolved oxygen level (DO) was higher than 6 mg DO/l, whereas, unionized ammonia concentration was lower than 0.2 mg NH_3/l throughout the study period. Total alkalinity and total hardness values ranged from 132 to 153 mg/l and 154-182 mg/l as $CaCO_3$, respectively. No significant differences were detected among the treatments in water quality parameters.

Growth performance (final body weight, weight gain and relative body weight gain) increased significantly ($P < 0.05$) when Nile tilapia fingerlings fed on *Ulva* diets supplemented with different treatments of the present study. Where data in table (4) and figure(1) showed that fish fed on T3 containing (FER + LC + PRO) and T6 containing normal *Ulva* in combination with (MEM + LC + PRO) showed higher average body weight, weight gain and relative body weight gain without significant difference between the two treatments.

The best ($P \leq 0.05$) survival rate (96.67 %) was observed when fish was maintained at the third diet containing (FER + LC + PRO) and the sixth diet containing normal ulva in combination with (MEM + LC + PRO) and there were no significance difference in survival rate between other treatments.

With respect to pooled means it is noticed that fermentation and Multienzymes treatments don't differ significantly in all parameters. However (LC + PRO) treatments were better than LC or PRO alone.

Results of Table (5) showed that fish fed on the third diet containing (FER + LC + PRO) and the last diet containing normal ulva in combination with (MEM + LC + PRO) consumed more feed (28.45 and 28.12 g feed /fish, respectively) than other treatments. Furthermore results showed that there were no significant differences in feed conversion ratio (FCR) and protein productive value (PPV %) between different experimental treatments.

Table (4): Growth performance parameters (means ± SE) of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Treat.		Initial	Final	Gain	DWG	SGR	Survival %
Ferm.	LC	5.12±0.00a	21.07±0.09b	15.95±0.09b	0.19±0.00b	1.68±0.00b	93.33±3.33ab
	PRO	5.15±0.01a	21.26±0.09b	16.11±0.09b	0.19±0.00b	1.69±0.01b	90.00±0.00b
	LC+PRO	5.13±0.02a	22.08±0.05a	16.95±0.07a	0.20±0.00a	1.74±0.01a	96.67±3.33a
MEM	LC	5.14±0.02a	21.06±0.26b	15.93±0.24b	0.19±0.00b	1.68±0.01b	93.33±3.33ab
	PRO	5.17±0.02a	21.27±0.19b	16.10±0.18b	0.19±0.00b	1.68±0.01b	93.33±3.33ab
	LC+PRO	5.15±0.02a	21.95±0.03a	16.81±0.04a	0.20±0.00a	1.73±0.01a	96.67±3.33a
Pooled Means:							
Ferm.		5.13±0.01g	21.47±0.16g	16.33±0.16g	0.19±0.00g	1.70±0.01g	93.33±1.67g
MEM		5.15±0.01g	21.43±0.16g	16.28±0.16g	0.19±0.00g	1.70±0.01g	94.44±1.76g
LC		5.13±0.01x	21.07±0.12y	15.94±0.12y	0.19±0.00y	1.68±0.01y	93.33±2.11y
PRO		5.16±0.01x	21.26±0.09y	16.10±0.09y	0.19±0.00y	1.69±0.00y	91.67±1.67y
LC+PRO		5.14±0.01x	22.02±0.04x	16.88±0.05x	0.20±0.00x	1.73±0.01x	96.67±2.11x

Means having the same letter in the same Colum is not significantly different (P<0.05).

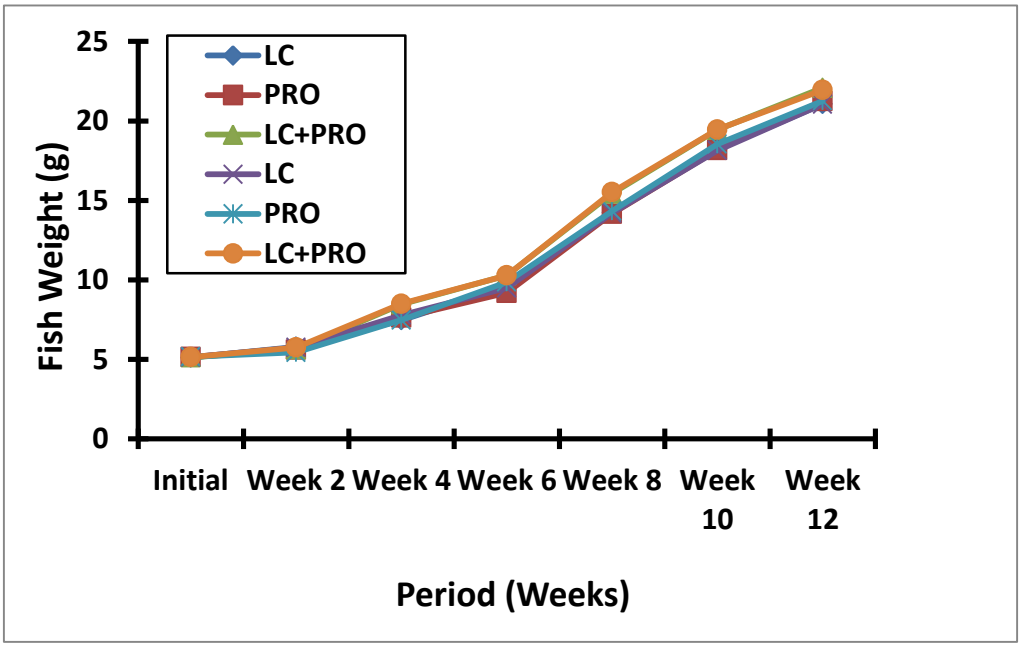

Figure (1): Changes in Nile tilapia live body weight (g)

Concerning with protein efficiency ratio (PER), results showed that highest PER value, 1.66 was obtained by fish fed fifth diet (MEM + PRO), followed by the fish maintained at third diet containing (FER + LC + PRO) 1.65 without any significant differences (P>0.05) between them .Pooled means parameters of feed utilization showed no significance difference among fermentation and MEM treatments

Table (5): Feed and nutrient utilization parameters (means ± SE) of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Treat.		Feed	FCR	PPV %	PER	ER %
Ferm.	LC	26.88±0.09b	1.69±0.01a	35.72±0.62a	1.63±0.54a	22.54±0.39a
	PRO	27.27±0.14ab	1.69±0.01a	32.67±2.07a	1.09±0.55b	24.27±2.25a
	LC+PRO	28.45±0.22a	1.68±0.01a	32.72±0.98a	1.65±0.53a	22.62±0.27a
MEM	LC	26.79±0.56b	1.68±0.01a	34.00±0.92a	1.08±0.55b	24.30±1.17a
	PRO	26.95±0.12b	1.67±0.01a	32.44±0.79a	1.66±0.55a	22.10±1.47a
	LC+PRO	28.12±0.12a	1.67±0.00a	33.42±0.97a	1.10±0.54b	25.13±1.32a
Pooled Means:						
Ferm.		27.53±0.25g	1.69±0.00g	33.70±0.85g	1.46±0.29g	23.14±0.72g
MEM		27.29±0.27g	1.68±0.01g	33.29±0.50g	1.28±0.29g	23.84±0.80g
LC		26.83±0.26y	1.69±0.01x	34.86±0.63x	1.36±0.37x	23.42±0.68x
PRO		27.11±0.11y	1.68±0.01x	32.56±0.99x	1.37±0.37x	23.19±1.29x
LC+PRO		28.29±0.13x	1.68±0.01x	33.07±0.64x	1.38±0.36x	23.88±0.82x

Means having the same letter in the same Colum is not significantly different (P<0.05).

The chemical compositions of whole body parameters of Nile tilapia fingerlings fed diets containing different experimental diets are summarized in Table (6). No significant differences were observed in fish moisture content and ash contents (P>0.05). Also there were no significance differences in total protein content among different experimental diets. Fish fed on fifth diet (MEM + PRO) had the highest levels for lipid content of fish (27.03%), however no significant difference in lipid content among third diet containing (FER + LC + PRO), the last diet containing normal *Ulva* in combination with (MEM + LC + PRO), first diet (FER + LC), and fifth diet (MEM + PRO). With respect to pooled means parameters of body composition showed no significance difference among FER and MEM treatments. The same trend observed for LC, PRO, (LC + PRO).

Table (6): Body composition (Means ± SE) of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Treat.		Fish Composition				
		Moisture	DM	CP	Lipid	Ash
Ferm.	LC	73.26±0.50a	26.74±0.50a	57.40±1.33a	26.60±2.25a	15.21±0.36a
	PRO	72.63±0.42a	27.37±0.42a	55.80±0.00a	22.84±1.76b	15.09±0.71a
	LC+PRO	72.48±0.61a	27.52±0.61a	57.25±0.20a	24.78±1.22ab	14.53±0.42a
MEM	LC	73.34±0.52a	26.66±0.52a	56.85±0.61a	22.45±1.71b	14.96±0.35a
	PRO	72.79±0.54a	27.21±0.54a	56.90±0.00a	27.03±1.28ab	15.08±0.78a
	LC+PRO	72.75±0.6a	27.25±0.60a	54.90±0.12a	24.14±1.31ab	15.37±0.50a
Pooled Means:						
Ferm.		72.79±0.28g	27.21±0.28g	56.82±0.46g	24.74±1.05g	14.94±0.28g
MEM		72.96±0.29g	27.04±0.29g	56.22±0.37g	24.54±0.98g	15.14±0.29g
LC		73.30±0.32x	26.70±0.32x	57.13±0.66x	24.53±1.57x	15.08±0.23x
PRO		72.71±0.31x	27.29±0.31x	56.35±0.25x	24.94±1.35x	15.08±0.47x
LC+PRO		72.62±0.39x	27.39±0.39x	56.08±0.54x	24.46±0.81x	14.95±0.35x

Means having the same letter in the same Colum is not significantly different (P<0.05).

Data in Table (7) showed that dietary supplementation of third diet containing (fermented ulva,Lcarinitine,probiotic) caused a significant increase in fulton condition factor however the first , the fourth , the third and the six diet containing normal ulva in combination with (multienzymemixture, Lcarinitine,probiotic) showed no significance differences in fulton condition factor.

Calculations of economical efficiency of the present experimental diets are shown in Table (8). Feed price and feed cost to produce one kg fish gain of Nile tilapia fingerlings fed on *Ulva* diets supplemented with different treatments of the second experiment were similar, where feed price ranged from4.08 to 4.68LE /kg and feed cost ranged from6.89 to 7.82.

Table (7): Changes in Nile tilapia Fulton condition factor (K), hepato somatic index (HSI), and viscera somatic index (VSI) of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Treat.		Somatic Indices		
		FCF	**HIS**	**VSI**
Ferm.	LC	1.70±0.04a	1.88±0.09b	8.67±0.62ab
	PRO	1.55±0.02b	2.15±0.38ab	9.26±1.73a
	LC+PRO	1.65±0.04a	2.35±0.60a	7.57±0.47b
MEM	LC	1.61±0.05ab	2.41±0.30a	7.72±0.41b
	PRO	1.59±0.05b	2.23±0.37a	10.10±0.36a
	LC+PRO	1.77±0.13a	2.29±0.84a	8.44±0.33ab
Pooled Means:				
Ferm.		1.63±0.03g	2.13±0.22g	8.50±0.60g
MEM		1.65±0.05g	2.31±0.28g	8.76±0.40g
LC		1.65±0.04x	2.14±0.18y	8.20±0.39y
PRO		1.57±0.03x	2.19±0.24y	9.68±0.81x
LC+PRO		1.71±0.07x	2.32±0.46x	8.01±0.32y

Means having the same letter in the same Colum is not significantly different (P<0.05).

Amino acid profile of studied fish:

17 amino acids were detected in amino acid profile of studied fish including essential and nonessential amino acids (Table 9).

With regard to essential amino acids, the most abundant amino acid for first diet (Ferm+LC), second diet (Ferm+Pro), third diet (Ferm+LC+ Pro) fourth diet (MEM+LC), and fifth diet (MEM + PRO) and six diet (MEM+LC+ PRO) were Lysine (3.53, 3.76, 3.68 , 3.89, 3.63 and 3.49 % respectively), Leucine (3.17, 3.34, 3.39, 3.57, 3.31and 3.27 %, respectively), Valine (2.34, 2.37, 2.48, 2.54, 2.43and 2.38 %, respectively) and Therionine (2.05, 1.89, 2.09, 2.09, 2.1 and 2.09 %, respectively)

On the other hand for NEAA, the major amino acid for first diet (FER+LC), second diet (FER+PRO), third diet (Ferm+LC+ Pro) fourth diet (MEM+LC), and fifth diet (MEM + PRO) and six diet (MEM+LC+ PRO) were Glutamic acid (7.69, 7.21, 7.56,

7.49, 7.06 and 6.8 %, respectively), Glycine (4.08,3.66, 4.09, 4.11, 3.93 and 4.03%, respectively), Aspartic (4.25, 4.39, 4.45, 4.73, 4.51and 4.31 %, respectively) and Alanine (3.7, 3.59, 4.03, 3.97, 3.57and 3.57 %, respectively).

Table (8) Economic efficiency for production of one kg gain of Nile tilapia (*Oreochromis niloticus*) fry fed at different experimental diets.

Treat.		**Economic efficiency**		
		Price/ kg feed (L.E)	**FCR (kg feed/kg gain)**	**Feed cost/kg gain(L.E)**
Ferm.	**LC**	4.08	1.69	6.89
	PRO	4.36	1.69	7.37
	LC+PRO	4.51	1.68	7.58
MEM	**LC**	4.25	1.68	7.14
	PRO	4.53	1.67	7.57
	LC+PRO	4.68	1.67	7.82
Pooled Means:				
Ferm.		4.32	1.69	7.28
MEM		4.49	1.67	7.51
LC		4.17	1.68	7.02
PRO		4.45	1.68	7.47
LC+PRO		4.60	1.68	7.70

Table (9) Nile tilapia amino acid profile of the second experiment.

Treatments	Ferm.+LC	Ferm.+PRO	Ferm.+LC+PRO	MEM+LC	MEM+PRO	MEM+LC+PRO
Essential amino acids (EAAs)						
Isoleucine	1.91	1.98	2.08	2.08	1.92	1.91
Therionine	2.05	1.89	2.09	2.09	2.1	2.09
Valine	2.34	2.37	2.48	2.54	2.43	2.38
Phenylalanine	1.79	1.93	1.87	1.87	1.83	1.77
Lysine	3.53	3.76	3.68	3.89	3.63	3.49
Leucine	3.17	3.34	3.39	3.57	3.31	3.27
Methionine	1.32	1.5	1.41	1.55	1.53	1.48
Histidine	1.11	1.28	1.2	1.1	1.03	1.02

Total EAAs	17.04	18.05	18.2	18.69	17.78	17.41
Non-Essential amino acids (NEAAs)						
Argnine	2.87	2.88	3.06	3.07	2.86	3.06
Aspartic	4.25	4.39	4.45	4.73	4.51	4.31
Alanine	3.7	3.59	4.03	3.97	3.57	3.57
Proline	2.83	2.58	2.64	2.52	2.62	2.86
Glutamic acid	7.69	7.21	7.56	7.49	7.06	6.8
Glycine	4.08	3.66	4.09	4.11	3.93	4.03
Serine	1.94	1.51	1.97	1.77	2.01	1.83
Cystine	0.4	1.26	0.46	1.13	0.49	0.61
Tyrosine	1.45	1.61	1.55	1.54	1.62	1.49
Total NEAAs	29.21	28.69	29.81	30.78	28.13	28.56

DISCUSSION

All values of the water quality parameters measured were within the acceptable range for the normal growth of tilapia as mentioned by Boyd (1984).

Results of the present study revealed that fish fed on the third diet (FER + LC + PRO) and the last diet (MEM + LC + PRO) showed higher significantly average body weight, weight gain and relative body weight gain without significant difference between the two treatments.

Given the potential complimentary modes of actions of exogenous digestive enzymes, probiotic, and L-carnitine or fermentation, probiotic and L-carnitine (when used in combination) could offer more benefits than when used alone. This is confirmed in this study with improved growth performance in terms of FBW, SGR, and FCR and PER observed in tilapia fed diet supplemented with combination of enzymes, probiotic and L-Carnitine or fermentation, probiotic, and L-Carnitine.

Results of the present study confirmed the benefits of exogenous enzymes preparations and its ability to enhance fish growth performance which could be attributed to their role in hydrolyzing connective tissues and skin where it is difficult for fish to digest broken down fiber and certain carbohydrates found in protein sources from grains and oil seeds. Also, they improve the activities of endogenous enzymes and the efficacy of the digestion process **(Hlophe-Ginindza *et al.*, 2016)**, protein and amino acids digestibility and growth performance **(Vielma *et al.*, 2004)** and reduce viscosity of NSP **(Bedford and Cowieson, 2012).**

The beneficial effects of probiotics on fish growth performance and feed utilization due to modifying the fish-associated (or ambient) microbial community of the gastrointestinal tract, thus promoting better feed utilization, enhancing the fish response towards disease and improving the quality of its ambient environment **(Verschuere *et al.*, 2000)**.

In addition L-carnitine is an effective means to augment the provision of lysine and methionin, the most limiting amino acids in plant-based diets. Where, L-carnitine is biosynthesized from lysine and methionine **(Steiber *et al.*, 2004)**, but the amounts of both amino acids are usually limited in the plant protein sources used in feeds **(Tacon and Jackson 1985)**. Therefore, the level of L-carnitine should be taken into account with respect to lipid metabolism, when fish meal in diets is substituted mostly or entirely by plant proteins. L-Carnitine plays an important role in lipid b-oxidation to facilitate the importation of activated long-chain fatty acids into mitochondria and the accompanying intermediate compounds out of the mitochondrial matrix **(Harpaz, 2005)**. Because of its role in lipid metabolism in fish, dietary carnitine supplementation has been found to enhance protein synthesis and promote growth performance **(Ozorio *et al.*, 2001)**. Although carnitine is widely distributed in cells, an animal-derived meal generally contains 10-20 times more L-carnitine than plant derived feedstuffs.

Moreover, fermentation might increase protein content, reduce peptide size, and eliminate anti-nutritional factors, such as trypsin inhibitors, non-starch polysaccharides (NSP) and phytates, thus making SM more acceptable to animals (**Hong *et al.*, 2004**).

Our obtained results are in accordance with **Hlophe-Ginindza *et al.* (2015)** who observed significantly improved growth performance in tilapia (*Oreochromis mossambicus*) when an exogenous enzyme cocktail, Natuzyme® (containing protease, lipase, α-amylase, cellulase, amyloglucosidase, β-glucanase, pentosonase, hemicellulose, xylanase, pectinase, acid phosphatase and acid phytase) was added to a plant based diet. Also **Adeoye *et al.* (2016)** reported that supplementation of the diet with a combination of enzymes (containing phytase, protease and xylanase) and probiotic (containing *Bacillus subtilis*, *Bacillus licheniformis* and *Bacillus pumilus*) is capable of improving tilapia growth.

A similar enhancement of growth performance with probiotic addition was reported in Nile tilapia supplemented with *Enteroccus faecium* (**Wang *et al.*, 2008a**), *S. cerevisiae* **(Abdel-Tawwab *et al.*, 2008; Goda *et al.*, 2012)** and with bacterial cocktail of (*Lactobacillus acidophilus, Streptococcus thermophilus, and Bifidobacterium bifidum*) **(Ayyat *et al.*, 2014)**.

Moreover, **Amer and El-Tawil (2011)** showed that final body weight (FBW), weight gain (WG) and specific growth rate (SGR%/day) of fish maintained at 10% seaweed (SW) or contained 0.5% probiotic (P) were more better significantly than fish maintained at control diet or (SW + P) diet. **Dowidar *et al.* (2017)** used three types of probiotics, multi-strain probiotics (6×107 cfu/g), *Bacillus subtilis* (1×1011 cfu/g) and *Saccharomyces cerevisiae* (2.6×1010 cfu/g) in Nile tilapia diet, and showed that the growth performance of fish fed different probiotics was higher than those fed the control diet.

On the contrary, **Iwashita *et al.* (2015)** mentioned that administration of a combination of probiotics of *B. subtilis, S. cerevisiae and A. oryzae* had no effect on the growth rates of Nile tilapia.

similar resuts of L-carnitine improvement effect recorded by **Amer *et al.* (2015)** who reported that supplementation of (LC) in fish diets enhanced fish performance over the plant diet control, where Fish fed on FM-based diet and SBM-based diet + 450 mg LC/kg diet recorded Highest final body weight followed by SBM-based + 300 mg LC/kg diet. These results supported by **Agouz *et al.* (2016)** revealed that dietary L-carnitine at any level significantly increased all growth parameters (body weight, body length, weight gain, specific growth rate).

Similar positive results of fermentation obtained by **Felix and Alan Brindo (2014)** who used raw and fermented *Ulva lactuca* as feed ingredients in giant freshwater prawn *Macrobrachium rosenbergii* diets *a*t three levels, 10, 20 and 30 % in diets. The best performance of prawn in terms of percentage weight gain, SGR was observed in the diets containing fermented *U. lactuca* than raw *U. lactuca*.

Fermentation techniques have advantages for higher inclusion of plant proteins instead of fish meal through inactivation of component Anti- Nutritional Factors, ANFs (Reddy and Pierson, 1994) as well as an increased range of low molecular weight proteins and peptides with potentially higher digestibility **(Kader *et al.*, 2012)**.

Our results revealed that the best survival rate was observed when fish was maintained at the third diet (FER + LC + PRO) and the last diet containing normal *Ulva* in combination with (MEM + LC + PRO) and there were no significance difference survival rate between other treatment. The improved survival rate may be due to the combined effect of combination of enzymes, probiotic and L-carnitine or fermentation, probiotic, and L-carnitine.

The present results confirm those obtained by **Amer *et al.* (2015)** who indicated that Survival rate improved significantly with all plant-based diets with L-carnitine at 300 mg/kg of Nile tilapia.

Results of the present study showed that fish fed on the third diet and sixth diet consumed more feed than other treatments. The significant differences of FCR suggest that the improved in growth related to feed consumption and better feed utilization efficiency may be due to enhancing the release of nutrients of plant-based diets by breaking down the bonds of phytate-protein and phytate-minerals complexes by dietary supplemented enzymes **(Vielma *et al.*, 2004)**. Therefore, the exogenous multi-enzymes may enhance the palatability of the plant diets.

With respect to PER, PPV%, and ER %, data indicated that highest PER value was obtained by fish fed fifth diet (MEM + PRO), followed by the fish maintained at third diet containing (FER + LC + PRO) without any significant differences between them. The beneficial effects of probiotics on PER, PPV%, and ER % appear to be associated with colonization of favorable microbiota in the gut which produce enzymes that

hydrolyze complex molecules facilitate better digestion and absorption of macronutrients resulting in higher protein and energy retention in the body **(Essa *et al.*, 2010)**. Similar positive results of multi enzymes mixture were found in study of **Lin *et al.* (2007)** who indicated that the highest apparent protein retention was observed in in juvenile hybrid tilapia fed the diet containing the 1.5 g /kg of enzyme complex (neutral protease, b-glucanase and xylanase).

Also, probiotics role appear in study of **Amer and El-Tawil (2011)** where they reported that the best significant values of feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV %), and energy retention (ER %) were found with fish maintained at T_2 diet contained 10% seaweed, T_3 diet contained 0.5% probiotic (P) and T_4 diet contained both of 10% seaweed and 0.5% probiotic, while control diet had the lowest significant values.

With regard to L-carnitine, **Amer *et al.* (2015)** demonstrated that fish fed on FM-based diet and SBM-based diet + 450 and 300 mg LC/kg diet had the best feed conversion ratio, protein productive value, and energy retention. Also **Agouz *et al.* (2016)** indicated that dietary L-carnitine at any level significantly improved feed conversion and protein efficiency ratio of Nile tilapia.

The same findings were observed by **El-Sayed *et al.* (2010)** who revealed that dietary LC has significantly enhanced the growth, PER and PPV of Nile tilapia despite the reduction of dietary protein from 30% to 20%. Although it is not clear how L-carnitine affects fish growth it is generally assumed that L-carnitine enhanced energy utilization through promotion of fatty acid oxidation and accordingly, sparing dietary protein for somatic growth. Thus the improvement in protein utilization results in an increase in growth **(Nekoubin *et al.*, 2012)**.

When talking about fermentation **Felix and Alan Brindo (2014)** used raw and fermented *Ulva lactuca* as feed ingredients in giant freshwater prawn *Macrobrachium rosenbergii* diets *a*t three levels, 10 %, 20 % and 30 % in diets. The best FCR was observed in prawn fed with FU at 20 % incorporation.

Elmorshedy (2010) reported that there were positive trends between protein efficiency ratio (PER) and energy retention (ER %) on one side and seaweeds inclusion levels in the diet up to the level of 14% on the other side. Also, **Diler *et al.* (2007)** stated that PPV% improved significantly with increasing dietary *Ulva* inclusion rate up to 15%.

The body composition of fish is primarily influenced by diet composition, feeding practices, fish size, and can be controlled through nutrition **(Burtle, 1990)**. However, Data of the present experiment explained that, no significant differences were detected among different fish groups for whole body composition as DM, protein, ash contents (%).

These results supported by **Ng and Chong (2002)** who indicated that exogenous enzyme Superzyme® enzyme (cocktail of xylanase, amylase, cellulase, protease and β-glucanase enzymes) supplementation in the diets did not have any effect on the whole

body composition of tilapia. Similary **Lin *et al.*, (2007)** demonstrated that no significant differences were detected among different fish groups for whole body composition as DM, protein, ether extract, ash contents (%) and gross energy by Nutrasexylam© addition. our results agree with these of **Amer and El-Tawil (2011)** who indicated that there were insignificant differences in fish body moisture, crude fat and crude protein contents among T_1 treatment which were without seaweed or probiotic supplements (control); T_2 treatment contained 10% seaweed (SW); T_3 contained 0.5% probiotic (P) and T_4 contained both of 10% seaweed and 0.5% probiotic (SW + P). Also, **Khalafalla and El-Hais (2013)** reported that enzyme complex (neutral protease, b-glucanase and xylanase) in juvenile hybrid tilapia diets had no significant differences on whole body moisture, protein, lipid and ash. Similar trend was detected by **Mahmoud *et al.* (2014)** who indicated that substituting FM with SBM did not affect the moisture, ash and gross energy. While crude protein was significantly higher in fish fed SBM-based diet supplemented with phytase compared to the FM-based diet. Also**, Amer *et al.* (2015)** concluded that FM can be completely replaced by SBM in Nile tilapia diets by the inclusion of L-carnitine at 300 g/kg without any significant differences in body composition. Moreover**, Amer (2017)** didn't find any significant difference in whole body composition of Nile tilapia fry fed diets supplemented with exogenous multi-enzyme preparation.

Concerning similar results of fermentation **Felix and Alan Brindo (2014)** used raw and fermented *Ulva lactuca* as feed ingredients in giant freshwater prawn *Macrobrachium rosenbergii* diets *a*t three levels, 10 %, 20 % and 30 % in diets and found that The whole body composition of prawns fed the raw and fermented *Ulva lactuca* incorporated diets did not show any variations in moisture, protein, lipid and ash.

Agouz *et al.* (2016) found that, as dietary L-carnitine increased fat content of tilapia fish significantly decreased and protein content significantly increased, while moisture and ash content did not significantly affected. The higher protein content and lower fat content were recorded with fish fed the diet contained the higher L-carnitine level (1200 mg/kg diet). **Opiyo *et al.*, (2019)** cited that fish fed on probiotics supplemented diets had significantly higher protein content and lower lipid content compared to the control.

Condition factor (K) factor is a measure of relative muscles to bone growth and the variation in the growth of these tissues due to diet treatment may be reflected by changes in K (**Ostrowski and Garling, 1998**). Moreover, K provides a measure of fish fitness (**Power, 1990**) and it is assumed that K reflects not only fish characteristics of the environment such as water quality, food availability, and habitate quality (**Liao *et al.*, 1995**).

Results of the present study showed that dietary supplementation of third diet containing (FER + LC + PRO) caused a significant increase in fulton condition factor while there were no significance differences in fulton condition factor among first ,

fourth , third and last diet containing normal *Ulva* in combination with (MEM + LC + PRO).

In this regards, **Lin *et al.* (2007)** used enzyme complex (neutral protease, b-glucanase and xylanase) in juvenile hybrid tilapia diets, and found that there were no significant differences in condition factor among dietary treatments. While Viscera ratio and hepatosomatic index decreased significantly with increasing enzyme.

Also**, Adeoye *et al.* (2016)** showed that diet supplemented with combination of enzymes (containing phytase, protease and xylanase) and probiotic (containing Bacillus subtilis, Bacillus licheniformis and Bacillus pumilus) did not affect somatic indices.

Amer *et al.* (2015) showed that inclusion of 1.5 g Natuzyme® in SBM-based diet reduced feed cost by 17.55% compared to the FM-based diet. That would save 8.78% of fish culture costs because feeding cost accounts for over 50% of production costs of aquaculture.

Our obtained results showed that feed price and feed cost to produce one kg fish gain of Nile tilapia fingerlings fed on Ulva diets supplemented with different treatments of the present study were similar. These results may be due to using all-plant diets in all treatments and the similar cost and effect of fermentation process and dietary exogenous enzymes supplementation on fish growth.

These observation supported by **Amer *et al.* (2012)** who revealed that plant diet supplemented with L-carnitine 300 mg /kg diet provides the fish culturist to save 0.18 kg feed per kg body weight gain, which is 9.84% less than the feed required for the SBM-based diet group. Also inclusion of L-carnitine in SBM-based diet+300 LC mg/kg diet reduced feed cost to produce 1 kg of fish gain by 26.73% compared to the fishmeal-based diet.

CONCLUSION

Considering these findings, we concluded that using fermented Ulva and inclusion of exogenous digestive enzymes in combination with L-Carnitine and/or probiotic is capable of improving growth performance and feed utilization of Nile tilapia. Also, it is economic and sharply reduced the fish feed cost.

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