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Evaluation of Rocket Leaves (*Eruca sativa*) as a Feed Additive on Growth Performance of Nile Tilapia, *Oreochromis niloticus,* Challenged with *Aeromonas jandaei*

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ABSTRACT

This study was carried out to evaluate the use of dry rocket leaves meal (DRLM); *Eruca sativa* as a natural feed additive on growth performance, feed utilization, body composition and innate immunity of Nile tilapia, *Oreochromis niloticus*. Four isonitrogenous (30% crude protein) and isocaloric (4.40 kcal/g) diets were formulated to contain 0.0 (control), 0.5, 1.0, and 1.5% DRLM. Fish (5.05 ±0.004 g) were distributed at a rate of 15 fish per 140-L aquarium in triplicates and fed tested diets at a rate of 5% at the first four weeks and 3% of live body weight at the rest of twelve weeks. The DRLM supplementation enhancement fish growth over the control diet and the highest fish performance was obtained at 1% DRLM level. There was no significant change in fish survival among the different treatments and its range was 93.3 - 95.6% suggesting that DRLM had no toxic effect. In regard to body composition, protein content in fish body was higher, while lipids contents were lower in fish fed DRLM diets than that fed the control diet. Blood profile showed an improvement in hemoglobin (Hb), red blood cell (RBCs), packed cell volume (PCV), and total protein, while aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels decreased in fish fed 1% DRLM. Supplementation of DRLM was found to have an antibacterial activity antagonistic to pathogenic bacteria *Aeromonas jandaei* infection in fish. Also, lysozyme and antibacterial activities increased significantly in DRLM fed fish. It could be recommended to use 1% DRLM as a feed additive for Nile tilapia to enhance its growth performance, health and innate immunity.

Keywords: Nile tilapia, dry rocket leaves, growth performance, feed utilization, body composition, biochemical parameters, innate immunity.

Introduction

Rocket plant (*Eruca sativa*) belongs to *Brassicaceae* family, has been grown in the Mediterranean area since Roman times and it is being presently extensively cultivated in various places for commercial purposes. Recently, rocket has gained greater importance as vegetables and culinary herb, especially among Middle Eastern and European populations (Alqasoumi *et al*., 2009; Lamy *et al*., 2008). Rocket (*E. vesicaria* subsp. *sativa*) contains a range of health-promoting phytochemicals including carotenoids, vitamin C, fibres, polyphenols, and glucosinolates (Villatoro-Pulido *et al*., 2012). Previous studies have shown as rocket species are rich in glucosinolates (Bennett *et al*., 2007; D'Antuono *et al*., 2008). Glucosinolates were found to have several biological activities including anticarcinogenic, antifungal, and antibacterial plus its antioxidant action (Kim *et al*., 2004). Glucosinolates are hydrolyzed by myrosinases in to different degradation products with a variety of biological activities (Chen and Andreasson, 2001).

Nile tilapia (*Oreochromis niloticus*) is a well-known tropical fish native to Africa. It is principally herbivorous, although occasionally omnivorous and it is an efficient converter of waste foodstuff and appears to thrive well on artificial supplemental feed (Omoregie *et al*., 2009). Nile tilapia farming is socially more acceptable and it is technically and economically more viable and sustainable. The attributes which make Nile tilapia so suitable for fish farming are its general hardiness and tolerance to a wide range of environmental conditions, ease of breeding, rapid growth rate, resistance to stress and disease, ability to efficiently convert a wide range of natural and artificial feed as well as organic and domestic wastes into high quality protein, ability to reproduce easily in captivity, and good taste (Osama, 2014).

Several efforts have been made to replace chemical drugs by herbal medicine in aquaculture industry (Citarasu *et al*., 2006; Obaroh and Achionye-Nzeh 2011).

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Motile *Aeromonads* are found in fresh and brackish water environments throughout the world and shows little or no host specificity. Fish pathogenic motile *Aeromonads* has been usually linked with *Aeromonase hydrophila, A. jandaei, A. zandali, A. sobria, A. ueronii and A. caviae* those may cause mass mortality in cultured fish (Rahman *et al.,* 2002). Generally, antibiotics were used to resist bacterial infection in fish but the residue of antibiotics may reach to the human. Therefore, attempts to use medicinal plants could be widely accepted as natural feed additives to enhance feed utilization, animal productivity, and innate immunity (Levic *et al*., 2008). From ancient times medicinal plants have been used due to their culinary qualities and medicinal properties including antioxidant activity and bacterial challenge (Tanabe *et al*., 2002 and Wangensteen *et al*., 2004). Therefore, this study was conducted to evaluate the use of dry rocket leaves meal as a natural feed additive on growth performance, feed utilization, body composition, biochemical parameters, and innate immunity of Nile tilapia challenged by pathogenic bacteria, *A. jandaei*.

Materials and Methods

1.1. Diet preparation and feeding regime:

Four experimental isonitrogenous (30% crude protein) and 7% lipid diets were formulated to contain 0.0 (control), 0.5, 1.0, and 1.5% DRLM. The fresh leaves of rocket were obtained from a local market and air-dried at room temperature. Dietary formulation and proximate composition of the experimental diets are shown in Table 1. The dry ingredients of each diet were thoroughly mixed, and 100 ml of water was added per kg diet. Afterwards, the mixture (ingredients and water) was blended using a kitchen blender to make a paste of each diet. Pelleting of each diet was carried out by passing the blended mixture through a laboratory pellet machine with a 1-mm diameter die. The pellets were dried in a drying oven for 24 hours at 65°C and stored in plastic bags in a refrigerator at −2°C until its use.

Table 1: Ingredients and proximate chemical analysis of the experimental diets (on dry matter basis) containing different levels of dry rocket leaves meal (DRLM).

Item	DRLM (%)			
	Control	0.5	1.0	1.5
Fish meal	12.4	12.4	12.4	12.4
Soybean meal	42	42	42	42
Ground corn	19	19	19	19
Wheat bran	14	14	14	14
Cod fish oil	2.9	2.9	2.9	2.9
Corn oil	2.3	2.3	2.3	2.3
Vitamins premix[1]	1.5	1.5	1.5	1.5
Minerals Premix[2]	1.5	1.5	1.5	1.5
Starch	4.4	3.9	3.4	2.9
Rocket leaves	0.0	0.5	1.0	1.5
Total	100	100	100	100
Proximate chemical composition				
Dry matter	90.9	91.7	91.8	91.3
Crude protein	30.2	30.2	29.7	30.9
Crude fat	8.2	8.3	8.2	8.3
Ash	9.5	9.4	9.2	9.1
Fiber	4.7	5.2	5.4	5.5
NFE[3]	47.4	46.9	47.5	46.2
GE(Kcal/100g)[4]	442.46	441.36	440.05	442.6
P/E ratio	68.25	68.42	67.49	69.81

[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 (NFE) = 100 – (protein% + lipid% + ash% + fiber%).

[4]- Gross energy (GE) was calculated from (NRC, 1993) as 5.65, 9.45, and 4.1 kcal/g for protein, lipid, and carbohydrates, respectively.

1.2. Fish culture technique:

Nile tilapia, *O. niloticus* (L.) were obtained from fish hatchery, Central Laboratory for Aquaculture Research, Abbassa, Abo-Hammad, Sharkia, Egypt. Fish were kept for two weeks in an indoor tank for acclimation during which were fed a formulated diet containing 30% crude protein. Fish were frozen at −20 °C for proximate analysis initially. Acclimated fish (5.05 ±0.004) g was distributed randomly in 12 140-L aquaria at a rate of 15 fish per each aquarium in triplicates. Each aquarium was supplied with compressed air via air-stones using aquarium air pumps. Settled fish wastes with one-half of aquarium's water were siphoned daily and water volume was replaced by aerated tap water from a storage tank. Fish were fed the tested diets at a feeding rate of 5% at the first four weeks and 3% of live body weight at the rest of twelve weeks. The diets were offered to each aquarium twice daily, for 12 weeks. Every two weeks, fish in each aquarium were group-weighed and the amount of feed was readjusted accordingly. The light: dark cycle during the running of this study was 14:10 h and water temperature range was 26.1-28.3°C

1.3. Growth performance and feed utilization:

Growth performance was determined and feed utilization was calculated as follows:
Weight gain = W2 – W1;
Specific growth rate (SGR) = 100 (Ln W2 – Ln W1) / T
Where W1 and W2 are the initial and final weights, respectively, and T is the number of days of the experimental period.
Feed conversion ratio (FCR) = feed intake / weight gain;
Protein efficiency ratio (PER) = weight gain / protein intake;
Apparent protein utilization (APU %) = 100 [protein gain in fish (g) / protein intake in diet (g)].
Energy utilization (EU %) = 100 [Energy gain in fish (g) / energy intake in diet (g)].

1.4. Hematological and biochemical parameters:

At the end of the experimental period, fish were not fed for 24 h prior to blood sampling. 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 two clean and dry tubes. The first part was centrifuged at 3,000 rpm for 15 min and the serum was stored at −20 ◦C for creatinin, aspartate aminotransferase (AST), alanine amino-transferase (ALT) and total protein analysis. The second part was mixed with EDTA solution for measuring hemoglobin (Hb), red blood cell (RBCs), and packed cell volume (PCV). Hb level was determined colorimetrically using spectrophotometer (Robonik An ISO 9001$ ISO 13485 company Made in India) and kit (Biomeria Made in France) according to Stopkopf (1983). PCV was determined using the microhaematocrit method Schalm (1975). RBCs were determined by using an Ao Bright-Line Heemocytometar according to the method described by Natt and Herrick (1952). Total protein content was determined colorimetrically using spectrophotometer (Robonik An ISO 9001$ ISO 13485 company Made in India) and kit (Biomeria Made in France) according to Henry (1964). Albumin and globulin were determined colorimetrically using spectrophotometer (Robonik An ISO 9001$ ISO 13485 company Made in India) and kit (Biomeria Made in France) according to Wotton and Freeman (1982). AST and ALT were determined calorimetrically using spectrophotometer (Robonik an ISO 9001$ ISO 13485 company Made in India) and kit (Biomeria Made in France) according to Rittman and Frankel (1975). Creatinine was determined colorimetrically using spectrophotometer (Robonik an ISO 9001$ ISO 13485 company Made in India) and kit (Biomeria Made in France) according to Henry (1974). Respiratory burst (Rb) and lysozyme was determined according to Siwicki (1989) and Schaperclaus *et al.* (1992).

1.5. Bacterial challenge:

At the end of the experimental period, fish of each treatment were divided into two subgroups; the first group was injected intraperitoneally (IP) with pathogenic *A. jandaei* (10^4 cell/ml), which was obtained from Fish Disease Department, Central Laboratory for Aquaculture Research, Egypt. The second group was injected IP with 0.2 ml of saline solution and used as a control. Both subgroups were kept under observation for 10 days post challenge during which incidences of daily mortality were recorded. Challenge test were determined according to Brook *et al*. (1988) and Miles and Misra (1983).

1.6. Proximate chemical analysis:

The tested diets and whole-fish body from each treatment at the beginning and at the end of the experiment were analyzed according to the methods of AOAC (1990) for moisture, crude protein, total lipids, and total ash. Moisture content was estimated by drying the samples to constant weight at 85 °C in a drying oven (GCA, model 18EM, Precision Scientic group, Chicago, Illinois, USA). Nitrogen content was measured using a microkjeldahl apparatus and crude protein was estimated by multiplying the nitrogen content by 6.25. Lipid content was determined by ether extraction in a multi-unit extraction Soxhlet apparatus for 16 h. Total 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 Sost (1970) and gross energy was calculated according to NRC (1993).

1.7. Statistical analysis:

The obtained data were analyzed by one way procedure (SPSS 2007) according to the following model $Y_{ij}= \mu+ T_i+ e_{ij}$, where μ: the over mean, T_i: the fixed effect of the rocket supplementation (1…4) and e_{ij}: random error. The differences between experimental groups were separated by Duncan's multiple range test (Duncan, 1995).

1.8. Economic analysis:

The cost of feed ingredients to produce a unit of fish biomass was estimated using a simple economic evaluation. The estimation was based on the local retail sale market price of all the dietary ingredients at the time of the study. These prices (in LE/kg) were as follows: herring fish meal, 12; soybean meal, 3.0; corn meal, 1.8; starch, 3; fish oil, 9; corn oil, 7; vitamin premix, 7.0; mineral mixture, 3; wheat bran, 3 and Rocket, 4.

Results and Discussion

The present study showed that fish fed on diet containing 1% DRLM showed the highest final weight, weight gain, relative gain%, and SGR in comparison to the experimental group (Table 2). The lowest final weight, weight gain, weight gain% and SGR were observed in fish fed on the control diet. Survival in all treatments was high and ranged from 93.3 to 95.6% indicating that DRLM has no toxic effect. Promoted growth in fish fed medicinal plants may be due to improving nutrients digestibility and growth-stimulant effect (Harikrishnan *et al*., 2011, Williams *et al.*, 2008 and Citarasu, 2010). This enhancement may be attributed to that DRLM contains a relatively large amount of vitamins B1, B2, C and pro-vitamin A, folic acid, glucosinolates, iodine, iron, protein, and especially calcium and sulphur compounds, which influence its characteristic odor, but also adds to its nutritional benefits (Palaniswamy *et al*., 2003). Moreover, Glucosinolates, the main active compounds of rocket leave, are hydrolyzed by myrosinases into different degradation products with variety of biological activities (Chen and Andreasson, 2001).

These results agree with Abd Elmonem *et al*. (2002) who studied the effect of different levels of roquette seed meal on growth performance and feed utilization of red tilapia fry. They formulated experimental diets to contain 0, 3, 6 and 9% of roquette seed meal instead of soybean meal. Red tilapia fed both meals exhibited better growth than fish fed the control diet, and the best growth were found at 3% roquette seed meal. Similarly, Soliman (2005) evaluated the nutritive value of using roquette seed (*E. sativa*) meal as a replacement for soybean meal in diets of Nile tilapia. He found that growth performance decreased significantly with increasing the substitution level of soybean meal with roquette seed meal. D'agaro (2006) stated that watercress (*Nasturtium officinale)* can be used to increase growth and survival in summer ling noble crayfish (A*stacus astacus*). Zeweil *et al*., (2008) study the effect of replacing three levels from rocket seed meal (RSM) as a partial or complete replacement of soybean meal protein of the control diet on growth performance, digestibility and blood characteristics in growing New Zealand White (NZW) rabbits. RSM contributed 0, 5, 10.5 and 21 % of the diet. They found that RSM at 10.5% level of the diet in NZW rabbits had the best results without adverse effects on growth performance. El-Nattat and ElKady (2007) indicated that 9% rocket seed meal in the diet gave the best final body weight compared to the control. Abd El Hakim *et al.* (2010) conducted an experiment with Nile tilapia fingerlings fed a basal diet containing 0, 1.0, 2.0, and 3.0% fennel, *Foeniculum vulgare* for 14 weeks. They found that the use of 1.0% fennel produced the maximum growth performance. Ahmad *et al.* (2011) evaluated the use of cinnamon, *Cinnamomum zeylanicum* (CSM) in a diet for Nile tilapia fingerlings for 12 weeks and they stated that 1.0% cinnamon is the optimum inclusion that gave the highest fish performance.

Table 2: Growth performance, feed utilization and survival of Nile tilapia fingerlings fed diets containing different levels of DRLM for 12 weeks**.**

Item	DRLM (%)		DRLM (%)		p
	Control (0.0)	0.5	1.0	1.5	
Initial weight (g)	5.05 ±0.004	5.07 ± 0.01	5.05 ± 0.01	5.07 ± 0.01	0.196
Final Weight (g)	21.05 ±0.15^{d}	23.17 ±0.10^{c}	25.17 ±0.15^{a}	24.17 ± 0.14^{b}	0.000
Weight gain (g)	15.99 ±0.14^{d}	18.05±0.10^{c}	20.11±0.16^{a}	19.10±0.13^{b}	0.000
Relative gain %	316.76 ±5.05^{d}	356.25±1.89^{c}	398.03±3.39^{a}	376.78±1.96^{b}	0.000
SGR (% / day)	1.70 ±0.01^{d}	1.81 ± 0.01^{c}	1.91± 0.01^{a}	1.86 ± 0.01^{b}	0.000
Feed intake	28.35 ±0.11^{c}	29.64± 0.21^{b}	30.91± 0.24^{a}	30.39± 0.26^{a}	0.000
FCR	1.77 ±0 .01^{a}	1.64± 0.002^{b}	1.53 ±0.001^{d}	1.59 ± 0.01^{c}	0.000
PER	23.13 ± 0.45^{c}	24.42±0.26^{b}	26.87± 0.27^{a}	24.52± 0.41^{b}	0.000
APU (%)	139.95±0.60^{d}	154.14±1.90^{c}	166.40±0.75^{a}	159.88±1.37^{b}	0.000
EU (%)	787.96±47.67^{b}	788.37± 44.15^{b}	952.09± 27.49^{a}	731.79±21.80^{b}	0.007
Survival rate (%)	93.3±3.58	93.3±3.58	95.6±2.22	93.3±3.58	0.000

Means the same letter in the same row is not significantly different at P<0.05.

Feed intake increased significantly, while FCR improved by using supplemented DRLM in fish diets (Table 2). Moreover, PER, APU and EU values increased significantly with increasing DRLM diets levels in diets. Increased feed intake resulted from the high demand for nutrients with stimulated growth or due to improved appetite because of sensory stimulation resulting from the presence of DRLM in the diets. The best FCR and higher values of FI, PER, APU, and EU were obtained when fish fed diet contained 1% DRLM. These results agree with Zeweil *et al*. (2008) who found that rabbits fed 10.5% RSM-diet were improved significantly in FI and FCR as compared to the control group. El-Nattat and ElKady (2007) indicated that 9% RSM in the diet gave the best final body weight and feed conversion ratio compared to the control. Abd Elmonem *et al*. (2002) studied the effect of different levels of roquette seed meal on growth performance and feed utilization of red tilapia fry. They formulated experimental diets to contain 0, 3, 6 and 9% of roquette seed meal instead of soybean meal. Red tilapia fed both meals exhibited better FCR, protein and energy utilization than fish fed the control diet, and the best growth were found at 3% roquette seed meal Ahmed *et al*. (2011) who showed that feed intake increased significantly, while FCR decreased significantly (P<0.05) when fish fed CSM-supplemented diets as compared to that fed on a control diet. Also, they found that highest and the lowest FCR were obtained at 0.0 (control) and 10 g CSM/kg diet. Abdel-Tawwab *et al*. (2010) observed a growth-promoting influence of green tea on Nile tilapia and they reported that the optimum growth and feed utilization were obtained at 0.5 g/kg diet. Talpur (2013) showed that *Mentha piperita* diet in feed led to significantly improved survival, weight gain and FCR for treated groups over the control in Asian sea bass *Lates calcarifer*.

In regard to fish body composition, Table 3 shows that there were significant differences in moisture, protein, ether extract, and ash contents due to DRLM supplementation. Protein content in fish body was higher, while lipids were lower in fish fed DRLM supplemented diets than that fed the control diet.

Table 3: Body composition of fed diets Nile tilapia fingerlings containing different DRLM for 12 weeks.

Items	DRLM (%)				P
	Control	0.5	1.0	1.5	
Moisture	75.49±0.05^{a}	74.57±0.04^{b}	73.87±0.06^{c}	73.46±0.07^{d}	0.0001
Crude protein	58.51±0.23^{b}	59.98±0.23^{a}	59.81±0.13^{a}	59.58±0.13^{a}	0.001
Ether extract	22.79±0.13^{a}	21.37±0.12^{b}	21.26±0.15^{bc}	20.89±0.05^{c}	0.0001
Ash	16.40±0.04^{a}	15.66±0.07^{b}	15.09±0.04^{c}	14.97±0.04^{c}	0.0001

Means the same letter in the same row is not significantly different at P<0.05

The effect of different DRLM levels of on different hematological and biochemical parameters of Nile tilapia is shown in Table 4. Hb, RBCs and PCV increase significantly in all treatments over the control diet. Fish fed diet contained 1 % DRLM exhibited the highest Hb, RBCs and PCV in comparison with those fed on the other experimental diets. Increase Hb in this study demonstrated that oxygen supply increases consequently, reflecting beneficial health effect on fish. Additionally, AST and ALT decrease significantly than in the control diet. No significant difference was observed in creatnine among treatments. It is noticed that fish fed on 1% DRLM showed the optimum hematological and biochemical parameters comparing with the control group. These results agree with Zeweil *et al.* (2008) who stated that RSM at 10.5% level of the diet in NZW rabbits had the best results without adverse effects on kidney or liver function. Serum total protein and globulin of rabbits fed 10.5% RSM-diet were significantly higher than those fed the control diet, while serum albumin and total lipids were not significantly affected by different treatments. Kolawole *et al*. (2011) stated that one way to

distinguish the appropriate or inappropriate prescription of medical plants is the assessment of their effects on hematological and biochemical parameters in experimental animals. These results agree with Asadi *et al.* (2012) who showed that oral administration of 1% watercress extract for at least 21 days increase Hb but no significant alternations were observed in the number of erythrocytes and leukocytes as well as hematocrit values. In other words, oral administration of watercress extract may concentrate Hb in red blood cells of fish. Increase in Hb, PCV, and numbers of leucocytes and thrombocyte were reported in Nile tilapia (Shalaby *et al*., 2006) and hybrid tilapia (Ndong and Fall, 2011) fed with diet enriched by garlic. Similar to the present study, the increase of total protein and globulin were recorded in rainbow trout after feeding ginger and garlic (Nya and Austin, 2009 a, b), cumin seed oil and nettle extract (Awad *et al*., 2013). Binaii *et al.* (2013) provides a new perspective for the use of medicinal plant as supplementation to beluga (*Huso huso*) feed to improve hematological and immunological indices.

Table 4: Hematological and Biochemical parameters of Nile tilapia as affected by different levels of DRLM for 12 weeks.

Items	DRLM (%)				P
	Control	0.5	1.0	1.5	
Hb (g/dl)	4.17 ± 0.14^{c}	4.51 ± 0.12^{b}	4.95 ± 0.15^{a}	4.44 ± 0.11^{b}	0.000
RBC_S($*10^6$/cmm)	1.25 ± 0.004^{c}	1.32 ± 0.003^{b}	1.42 ± 0.006^{a}	1.32 ± 0.005^{b}	0.001
PCV(%)	11.63 ± 0.21^{c}	12.41 ± 0.024^{b}	13.68 ± 0.31^{a}	12.51 ± 0.18^{b}	0.001
T.protein (g\dl)	3.08 ± 0.04^{c}	3.17 ± 0.02^{b}	3.52 ± 0.05^{a}	$3.21\pm.06^{b}$	0.007
S.albumin (g\dl)	0.91 ± 0.02^{c}	1.13 ± 0.03^{b}	1.28 ± 0.06^{a}	1.15 ± 0.05^{b}	0.011
S.globulin	2.17 ± 0.02^{b}	2.04 ± 0.02^{c}	2.24 ± 0.03^{a}	2.06 ± 0.03^{c}	0.017
AST (U\L)	13.91 ± 0.22^{a}	12.13 ± 0.3^{b}	10.28 ± 0.6^{c}	12.25 ± 0.5^{b}	0.000
ALT (U/L)	11.82 ± 0.14^{a}	11.13 ± 0.3^{b}	9.28 ± 0.6^{c}	11.25 ± 0.5^{b}	0.000
S.creatinine (mg\dl)	0.28±0.02	0.27±0.03	0.26±0.06	0.27±0.051	0.008

Means the same letter in the same row is not significantly different at P<0.05

After bacterial challenge, fish mortality was 90% in fish fed the control diet and it was 30-40% in fish fed on DRLM diets (Table 5). The experimentally infected fish died with some clinical signs such as tail and fin rot, scale less with external skin hemorrhage, while the postmortem finding was septicemic lesions of the internal organs. *A. jandaei* was re-isolated from liver, kidneys and spleen of the moribund and recently dead fish. NBT assay was used to determine the activity of phagocytes especially neutrophils and monocytes. NBT activity increased significantly when fish fed 0.5, 1 and 1.5% DRLM compared with the control group which was 0 (Table 5). Lysozyme levels are a family of enzymes with antibacterial activity characterized by the ability to damage the cell wall of bacteria. Lysozyme level increased with 0.5, 1 and 1.5% DRLM compared to fish group fed with control diet (Table 5). So, significant increase in lysozyme activity in plasma of fish fed for 21 days with diet enriched with 0.5, 1 and 1.5% DRLM may indicate an increase the fish's defense system against bacterial infection. The results obtained in this study show that DRLM increased disease resistance and improved fish survival against *A. jandaei* experimentally infection, These results may be attributed to the presence of glucosinolates, which were found to have several biological activities including anticarcinogenic, antifungal, and antibacterial plus its antioxidant action (Kim *et al*., 2004). Further, Glucosinolates are hydrolyzed by myrosinases in to different degradation products with a variety of biological activities (Chen and Andreasson, 2001). Awad *et al*. (2013) examined the efficacy of dietary black cumin seed oil (*Nigella sativa*) and nettle extract (Quercetin) on the immune response of rainbow trout, *Oncorhynchus mykiss*. They reported that treated fish fed 1% Quercetin and 3% *N. sativa* oil showed highly significant differences in lysozyme, total protein, antiprotease and bactericidal activities. Therefore, they suggest that by using these supplements there will be an increase in the immune function of rainbow trout. These results are agreement with Basha *et al*. (2013), Park and Choi (2012).

Table 5: Change in fish mortality rate after bacterial challenge, respiratory brust and lysozyme activities of Nile tilapia with different DRLM levels.

Fish groups	Challenge test Mortality (%)	Respiratory burst (NBT) Activity	Lysozyme (µg/ml serum)
Control (0.0)	90	0.214	0.186
0.5%	40	0.253	1. 039
1%	30	0.309	1.576
1.5%	30	0.339	2.04

Economic evaluation of the experimental diets containing different DRLM as feed additives levels 0.0, 0.5, 1.0 and 1.5% DRLM are shown in Table 6. The reduction in feed cost compared with control diet showed 13.4 % to produce one kg fish gain in the of treatment containing 1% DRLM level. Previous studies showed that the use of spices in small amounts gave lower incidence cost and higher profit index of fish species (Abd Elmonem *et al.*, 2002; Shalaby *et al.*, 2003 and El-Dakar, 2004).

Table 6: Economic efficiency for production of one Kg gain of fingerlings Nile tilapia diets containing different levels of Rocket.

Items	Control- 0.0	**DRLM levels** %		
		0.5 %	1 %	1.5 %
Price/ kg feed P.T	4.21	4.22	4.22	4.23
FCR (kg feed/kg gain)	1.77	1.64	1.53	1.59
Feed cost / kg gain P.T	7.46	6.92	6.46	6.73
Reduction cost in kg gain	100	7.23	13.40	9.78

Conclusion:

This study was carried out to evaluate the use of dry rocket leaves meal *Eruca sativa* as a feed additive on growth performance, feed utilization, body composition and innate immunity of Nile tilapia. The dry rocket leaves meal enhancement fish growth rate, feed utilization, biochemical and hematological parameters and innate immunity. The highest fish growth performance was obtained when fish fed diet containing 1%.

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