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Effects of Garlic (*Allium sativum*) and Curcumin (Turmeric, *Curcuma longa* Linn) on Nile Tilapia Immunity

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Abstract

The present study aimed to investigate the effect of different concentrations of garlic and/or curcumin in fish diet on growth performance, total blood protein, albumin, globulin, phagocytic activity, phagocytic index and disease resistance against challenge with pathogenic bacterial agent *Pseudomonas fluorescens*. Fish were separated into six experimental groups of 0% (control),1% garlic, 2%garlic, 1% curcumin, 2% curcumin and (1% garlic plus 1% curcumin mixed group) fed at 3% body weight per day.

Results showed that garlic and curcumin supplementation have significant improvement on growth performance compared to control except the garlic and curcumin mixed group. Total protein and globulin have significantly increased in all treated groups than control, but both of total protein and globulin were dropped with increasing the concentration of either garlic or curcumin. Phagocytic activity and index were significantly higher in all groups than the control. The mortality rate after challenging with *Pseudomonas fluorescens* were decreased in all treated groups than the control except the garlic curcumin mixed group.

It could be concluded that inclusions of garlic or curcumin in *Oreochromis niloticus* (*O. niloticus*) diets improve the growth performance and immunity as was indicated by significant increase of total protein, globulin, phagocytic activity and index of phagocytes which enhanced the resistance of challenged fish to *Pseudomonas fluorescens*.

Keywords: Curcumin, Garlic, Growth, Immunity, *Oreochromis niloticus*.

Introduction

Currently, Egypt is one of the countries where aquaculture is growing fast. Nile tilapia is the most widely farmed species (FAO, 2005). Diseases outbreaks are major problem in aquaculture (Yunxia et al., 2001). The use and abuse of disinfectants and antibacterial have led to emergence of resistant bacteria and drug residues in treated fish (FAO/WHA/OIE, 2006).Immune-stimulants are now used to enhance the activites of non-specific defense mechanisms and increasing disease resistance in aquaculture (Raa1996).

Herbs could be taken as supplementary materials in aqua feed formulation (Poongodi et al., 2012). Garlic (Allium sativum) is one of the known medicinal plants used as immune-stimulant (Lewis and Elvin-Lewis, 2003). Harris et al., (2001) reported that garlic has antibacterial, antiviral, antifungal and antiprotozoal and also beneficial effects on the cardio-vascular and immune system. Garlic has been used to control pathogenic bacteria and fungi in animals including fish (Corzo-Martnez et al., 2007).Turmeric (Curcuma longa) is a perennial herb that grows to a height of three to five feet and is cultivated extensively in Asia and other countries with a tropical climate. Curcumin is an active ingredient from the spice. Turmeric or curcumin is a medicinal plant that belongs to the family Zingiberaceae. The rhizomes of turmeric contain a yellowish colouring matter called curcumin. Curcumin has been shown variety of properties related to health such as hepato-protective agent (Pal et al., 2001),anti-inflammatory, immunomodulating, tumor-preventing (Miquel et al., 2002) and antibacterial activity (Singh et al., 2002).Turmeric is used for wound healing, inflammation and acidity (Jyothi et al., 2003 and Kumar et al., 2006).Turmeric is potent antioxidant (El-Bahr et al., 2007 ; Salama et al., 2007).In aquaculture, the turmeric extract supplementation in feed is an interesting alternative to disease control by enhancing the immune system in several fish such as rohu (Swagatika et al., 2008), goldfish (Harikrishnan and Balasundaram, 2008; Harikrishnan et al., 2009) and marine shrimp such as Pacific white shrimp (Vanichkul et al., 2010).It would be beneficial to supplement synthetic curcumin analogue (salicyl-curcumin) along with the aquaculture feed in order to help the fish to cope with adverse conditions in the environment. This would increase the survival rate, disease resistance and ultimately the growth rate.

The present study was conducted to investigate the effects of garlic and/or curcumin in fish diet on growth performance, serum proteins, phagocytic assay and disease resistance against challenge with *Pseudomonas fluorescens*.

Material and Methods

Experimental Fish

A total of 120 apparently healthy *O. niloticus* with average body weight of 60 ± 5g/fish were obtained from a private fish farm at Kafr El-Sheikh governorate. Fish were transported a live to the laboratory of Animal health research institute at Kafr El-Sheikh. Fish were kept in glass aquaria (90 x 45 x 45 cm). These aquaria supplied with chlorine free tap water according to (Innes 1966). The aquaria were continuously aerated by electric pump and held at 25 ± 2°C and half of the water were changed daily. Fish was acclimatized for one week and during the acclimatization fish fed on the basal diet only.

Feeding diets and experimental design

The diets were formulated according to N.R.C. (1993) after chemical analysis of Sun dried garlic powder and *Curucuma longa* powder used in experiment according to the Association of official Analytical chemists (1984) (Table 1 and 2). Fish were randomly divided to six groups received the prepared pelleted experimental diet according to the experimental design (Table 3). The diet was daily provided at 3% of body weight as described by Eurell et al. (1978). The daily amount of food was offered on two occasions over the day (at 9 AM and 12 PM). Feed intake and body weight of fishes were recorded weekly for measuring growth performance. Body weight, body weight gain and feed conversion ratio were calculated according to Vohra; Roudybush (1971); Castell and Tiews (1980); Tacon (1987), respectively.

Table 1. Chemical analysis of feed additives used in experimental diet

	Dry Matter	Moisture	Crude Protein	Energy
Sun dried garlic powder	82.02	17.98	14.805	2407.5
Curucuma longa powder	91.50	8.50	9.95	2078

Table 2. Physical and chemical composition of the experimental diets

	Control	Garlic1%	Garlic 2%	Curcumin1%	Curcumin 2%	Garlic1% + Curcumin 1%
Sun dried garlic powder	-	1	2	-	-	1
Curucuma longa powder	-	-	-	1	2	1
Yellow corn	45.3	44.5	44.5	44.5	44.5	44.5
Soybean meal 44%	21.5	21	19	20.8	18.7	18.7
Glutin62%	15.7	16	17	16.2	17.3	17.3
Fish Meal 60%	15	15	15	15	15	15
Dicalcium phosphate	2.2	2.2	2.2	2.2	2.2	2.2
Premix **	0.3	0.3	0.3	0.3	0.3	0.3
Chemical composition	%	%	%	%	%	%
ME Kcal/kg	3004	3001	3018	3001	3016	3015
Crudeprotein	32	32	32	32.14	32	32
Calcium	1.3	1.3	1.3	1.4	1.4	1.3
Available phosphorus	0.5	0.5	0.5	0.5	0.5	0.5
Lysine	1.53	1.52	1.47	1.51	1.47	1.47
Methionine + cystine	1.17	1.17	1.17	1.17	1.77	1.17

** The used premix (*Multivita Co.*) composed of vitamin A 12000000 IU, vitamin D_3 2200000 IU, vitamin E 10000 mg, vitamin K_3 2000 mg, vitamin B_1 1000 mg, vitamin B_2 5000 mg, vitamin B_6 1500 mg, vitamin B_{12} 10 mg, Niacin 30000 mg, Biotin 50 mg, Folic acid 1000 mg, Pantothenic acid 10000 mg, Iron 30000 mg, Manganese 60000 mg, Copper 4000 mg, Zinc 50000 mg, Iodine 1000 mg, Cobalt 100 mg, Selenium 100 mg, calcium carbonate ($CaCO_3$) carrier to 3000g.

Blood collection:

At the end of the experiment, 2ml blood sample/fish via the caudal vessels were collected from 3 fish from each group of the experiment according to Hawak et al., (1965). One ml of blood was collected with syringe containing anticoagulant (Heparin) and used for phagocytic assay (Kawahara et al., 1991) and the another ml of blood was used for serum collection for biochemical determination (Lied et al., 1975). Serum total protein was determined according to Doumas et al., (1981). Serum

albumin was determined according to Reinhold (1953). Serum globulin was determined by subtract the total serum albumin from total serum protein according to Coles (1974) and Khalil (2000). Albumin/ globulin ratio was determined by devision of serum albumin value on serum globulin value according to Saffinaz (2001).

Challenge test:

At the 7th week ten fish from each group (bacteriologically tested and determined to be free from bacterial infection) were artificially infected by s/c injection with 3ml/fish of culture suspension of pathogenic *Pseudomonas fluorescens* previously adjusted to 10^4. Specificity of death was determined by re-isolation of injected bacteria from freshly dead fish during the period of observation (one week) according to Soliman (1988).

Statistical analysis:

The data were statistically analyzed according to (SAS, 1987).

Table 3. Applied experimental design according to feed additives level

Group	*Diet*
1	Basal diet (control)
2	Basal diet contain garlic powder 1%
3	Basal diet contain garlic powder 2%
4	Basal diet contain *Curucuma longa* powder 1%
5	Basal diet contain *Curucuma longa* powder 2%
6	Basal diet contain garlic powder 1% plus *Curucuma longa* powder 1%

Results

The analysis of variance indicated that garlic powder and Curucuma longa powder significantly improve live body weight; body weight gain and feed conversion ratio in all treatments except the group fed on diet contain combined mixture of both garlic and Curucuma longa powder when compared with control (Table 4). Growth performance measurements showed best results in group fed on diet contain Curucuma longa powder 1% followed by group fed diet contain Curucuma longa powder 2%, group fed on diet contain garlic powder 1% and group fed on diet contain garlic powder 2%, respectively. Meanwhile the groups fed on diet containing garlic powder 1% plus Curucuma longa powder1% showed no significant difference when compared with control. The

examination of serum revealed significant increase in total protein in all groups comparing to the control one but the Albumin /globulin ratio significantly changed in all groups in comparison to control except the garlic and curcumin mixed group (Table5). Phagocytic percentage and phagocytic index were significantly higher in all treated groups than the control (Table 6). Mortalities of O. niloticus challenged with Pseudomonas fluorescens were significantly lower in all treated groups than the control except the garlic and curcumin mixed group (Table 7).

Table 4. Summary of influence of garlic powder and/or *Curcuma longa* powder supplementation on growth performance of *Oreochromis niloticus* during experimental period

Growth measurements	Group					
	Control	Garlic1%	Garlic2%	Curcumin1%	Curcumin2%	Garlic1%+ Curcumin1%
Cumulative initial body weight	a 61.00±0.9	a 62.15±0.6	a 62.20±0.8	a 62.30±0.79	a 62.20±0.85	a 62.40±0.81
Cumulative final body weight	c 88.71±1.2	b 96.00±1.0	b 93.89±1.1	a 100.56±1.26	b 97.00±1.24	c 89.11±1.36
Cumulative total body gain	d 28.65±0.5	b 34.47±0.5	c 32.44±0.6	a 38.83±0.63	b 35.44±0.62	d 27.22±0.67
Cumulative average F.C.R.	a 1.60±0.12	d 1.41±0.11	c 1.48±0.13	e 1.30±0.12	d 1.38±0.12	a 1.72±0.11

Means within the same row of different letters are significantly different at ($P < 0.05$)

Table 5. Influence of garlic powder and/or *Curcuma longa* powder supplementation on serum proteins (total protein, Albumin, Globulin and Albumin/Globulin ratio) of *Oreochromis niloticus*

Item	Group					
	Control	Garlic1%	Garlic 2%	Curcumin1%	Curcumin 2%	Garlic1%+ Curcumin 1%
Total Protein	d 5.36±0.22	a 6.57±0.44	c 5.80±0.52	a 6.49±0.54	b 6.08±0.15	c 5.80±0.27
Albumin	c 2.96±0.09	a 3.73±0.37	a 3.58±0.25	a 3.75±0.44	b 3.46±0.09	b,c 3.16±0.16
Globulin	c 2.41±0.13	a 2.84±0.06	c 2.21±0.46	a,b 2.74±0.10	b 2.63±0.06	b 2.64±0.12
A/G Ratio	c 1.23±0.03	b 1.31±0.10	a 1.82±0.50	b 1.36±0.11	b 1.32±0.01	c 1.20±0.01

Means within the same row of different letters are significantly different at ($P < 0.05$)

Table 6.Influence of garlic powder and/or *Curcuma longa* powder supplementation on phagocytic activity and phagocytic index of *Oreochromis niloticus*

	Control	Garlic1%	Garlic2%	Curcumin1%	Curcumin 2%	Garlic1%+ Curcumin1%
Phagocytic Activity	d 15.17±0.31	b 20.00±0.97	b 20.50±0.34	a 24.33±0.33	a 24.00±0.26	c 17.17±0.31
Phagocytic Index	c 5.17±0.31	b 9.00±0.58	b 8.50±0.34	a 12.00±0.26	a 12.17±0.31	b 6.83±0.31

Means within the same row of different letters are significantly different at ($P < 0.05$)

Table 7. Influence of garlic powder and/or *Curcuma longa* powder supplementation on mortality percent after challenge with *Pseudomonas fluorescens*

Group	No.	Mortality		Protected	
		No.	%	No.	%
Control	10	10	100	0	0
Garlic powder 1%	10	8	80	2	20
Garlic powder 2%	10	4	40	6	60
Curcumin powder 1%	10	4	40	6	60
Curcumin powder 2%	10	8	80	2	20
Garlic powder 1%+ Curcumin powder 1%	10	10	100	0	0

Discussion

Natural immune-stimulants are considered promising alternatives to chemotherapy and vaccines because of their broad spectrum activity, cost-effectiveness and eco-friendly measures (Anderson 1992).

The present study indicated significant changes in growth performance of all *O. niloticus* garlic or curcumin treated groups in comparison to control. These results are similar to those observed by Nya and Austin (2009) in rainbow trout (*Oncorhynchusmykiss*, Walbaum) fed at 0.5 and 1.0g of garlic / 100g of fish feed and Lawhavinit et al., (2011) in White shrimp supplied with 15 g/kg of ethanolic turmeric extract. This result was supported also by those reported by Maniyan et al., (2009).

It would be beneficial to supplement synthetic curcumin analogue (salicylcurcumin) along with the aquaculture feed in order to help the fish to cope with adverse conditions in the environment. This would increase the survival rate, disease resistance and ultimately the growth rate. The obtained results are similar also to those obtained by Javandel et al., (2008). Diet containing the highest garlic meal dosage (2%) had a significantly lower daily weight gain in compare to other groups ($P<0.05$). It could be concluded that the weight gain reduction in groups fed 2% dietary garlic meal means that herbal additives have their limitations too and needs more investigation.

The present results illustrated that supplementation of 1% curcumin in *O. niloticus* feed improving the growth performance better than the garlic supplementation. These results disagree with those obtained by Poongodi et al., (2012) after supplementation of garlic, ginger, turmeric and fenugreek in diet of *Macrobrachiumrosenbergii* post larvae which may be related to the different fish species.

Certain herbal immune-stimulants have been reported to increase total protein as well as total globulin in fish (Rao et al., 2004). The total protein as well as total globulin levels were significantly higher in all treated groups than the control except in garlic 2% group. Similar results were also obtained after garlic supplementation in the diet of *O. niloticus* fingerlings by Shalaby et al., (2006) and Diab et al., (2008).

The level of total protein and globulin decreased with increasing in the concentration of garlic as well as curcumin. This is nearly similar to the results obtained by Agatha (2012) where the level of total protein dropped

with increasing in the concentration of garlic. Improvement of such biochemical parameters was also observed after turmeric supplementation in *Mugil cephalus* fish (El-Bahr and Saad, 2008) and in Rohu fish (Sahu et al., 2008). The results obtained in the present study differ from those obtained by Abdelwahab and El-Bahr (2012) where turmeric mixed with seeds of *Nigella sativa* and supplemented to Asian sea bass fingerlings and resulted in no changes in serum total protein, albumin or globulin values in the treated groups, this is may be due to mixing with *Nigella sativa* and/or the fish species and fish age.

Phagocytosis is a primary, non- specific defense mechanism against invasion of pathogenic organisms of hosts (Olivier et al., 1988).The present study showed significant increasing in the phagocytic percentage as well as phagocytic index in all treated group as compared to the control. The same results were obtained by Nya and Austin (2009) in rainbow trout (*Oncorhynchusmykiss*, Walbaum) fed at 0.5 and 1.0g of garlic / 100g of fish feed and Elkamel and Mosaad (2012) in *O. niloticus* fish supplied by *Nigella sativa* and/or *Bacillus subtilis*.

Inclusion of garlic or curcumin in *O. niloticus* feeding have positively impacted the resistance of fish to *Pseudomonas fluorescens* infection as was indicated by significantly lower mortality rates of fish challenged by *Pseudomonas fluorescens* in comparison to the control but mixing of 1% garlic and 1% curcumin give drastic mortality rate. This may be attributed to presence of antagonism between the two herbs. This point needs further studies. These results were nearly obtained by Diab et al., (2008) after challenging garlic supplemented *O. niloticus* fingerlings by *Pseudomonas fluorescens*.

Conclusion

It could be concluded that addition of garlic or curcumin in *O. niloticus* diets improve the growth performance and immunity as was indicated by significant increase of total protein, globulin, phagocytic activity and index of phagocytes which enhanced the resistance of challenged fish to *Pseudomonas fluorescens*. We noticed that the low dietary level (1%) from each of garlic or curcumin powder induced better effects than higher dietary level or combined mixture of both. So, combined mixture of the two famous traditional medicinal plants need more studies.

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تأثير استخدام الثوم والكركم علي الحالة المناعية لأسماك البلطي النيلي

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استهدف البحث دراسة تأثير التركيزات المختلفة المضافة في علف الأسماك لكل من الثوم أو الكركم أو تأثير الخلط بينهماعلي كل من : نمو الأسماك و معدل تحولها الغذائي, بالاضافة الى البروتين الكلي,الألبيومين, الجلوبيولين فى الدم, النشاط البلعمي, مؤشر البلعمة و مدي مقاومة الأسماك لميكروب السيدومونس فلورسنس بعد عمل اختبار تحدي بهذا الميكروب للأسماك الخاضعة للبحث. تم تقسيم الأسماك الي ست مجموعات بحثية وهي: المجموعة الضابطة, المجموعة الثانية و الثالثة وتحتوي كلا منهما علي مسحوق الثوم بنسبة 1% و 2% /كجم علف علي التوالي, أما المجموعة الرابعة و الخامسة فتحتوي كلا منهما علي مسحوق الكركم بنسبة 1% و 2%/كجم علف علي التوالي وأخيراً المجموعة السادسة والتي تحتوي علي خليط مسحوق كلا من الثوم بنسبة 1% والكركم بنسبة 1% /كجم علف. تم تقديم العلف للأسماك يوميا بنسبة 3% من وزن الأسماك. أظهرت النتائج أن إضافة كل من مسحوق الثوم أو مسحوق الكركم لعلف الأسماك أدي إلي تحسين نموها ومعدل تحولها الغذائي مقارنة بالمجموعة الضابطة عدا المجموعة السادسة والتي تحتوي علي خليط مسحوق كلا من الثوم بنسبة 1% والكركم بنسبة 1% /كجم علف, كما أوضحت النتائج أيضا زيادة كل من البروتين الكلي و الجلوبيولين في كل المجموعات الخاضعة للبحث مقارنة بالمجموعة الضابطة و لكن هذه الزيادة كان يقل معدلها مع زيادة تركيز الثوم أو الكركم.النشاط البلعمي ومؤشر البلعمة كان مرتفعا في كل المجموعات موضع الدراسة بالمقارنة بالمجموعة الضابطة.النتائج بينت أن نسبة النفوق في الأسماك في كل المجموعات كانت أقل من المجوعة الضابطة عدا المجموعة السادسة وذلك بعد إجراء اختبار التحدي الذي خضعت له كل المجموعات البحثية. مما سبق نجد أن هذه الدراسة توضح أن إضافة مسحوق الثوم بنسبة1% أو إضافة مسحوق الكركم بنسبة 1% مع عدم الخلط بينهما في أعلاف أسماك البلطي النيلي يحسن من كفاءة النمو والأداء المناعي لهذه الأسماك مما يزيد مقاومتها للأمراض.