EFFECT OF SANGUINARINE AND VITAMINS AD_3E ON GROWTH PERFORMANCE IN *CYPRINUS CARPIO*

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ABSTRACT

A preliminary study was conducted to evaluate the effect of dietary supplementation of commercial product containing the isoquinoline alkaloid sanguinarine (Sangrovit®) by 50 g/ton ration, and Vitamins AD_3E (Baytara®) by 2 ml/Kg ration on common carp (*Cyprinus carpio*) growth performance, haematological parámeters, gut microbiota, biochemical parameter, and immunological parameter when infected with *Aeromonas hydrophila* and histopathological finding compared to the control group determined at 15 days intervals during the feeding trial. Each dietary treatment had two replicate aquaria. The results showed that during the period of experiment using these feed additives showed mild significant increase in growth performance (Weight gain, Average daily gain, Feed intake and Feed conversion ratio), except in sanguinarinE plus vitamins which showed highly significant ($P<0.05$) increase specially at 45 days, meanwhile all groups showed non significant changes in Erythrogram and Leucogram except in sanguinarine supplemented group, there was mild increase. All groups showed significant ($P<0.05$) decrease in gut micobiota (total bacterial and coliform count) and also more resistance to challenge with *Aeromonas hydro-phila.* In liver, kidney function and serum protein there were non significant changes along all periods except after infection with *Aeromonas hydrophila,* there where mild changes in these biochemical functions so the feed additives enhance the fish defense mechanism and elevates the responsibility of fish to disease.

INTRODUCTION

Fish play a vital role as a source of animal protein worldwide and increasing role in solving the human nutritional problems in Egypt. Fortunately, there are different and extensive water sources that are expected to yield tremendous amounts of fish in Egypt. Also, fish breeding and fish cultures are considered good ways to increase the consumption need from animal protein and major source of income.

Bacterial diseases among cultured

fish either primarily or secondarily are considered to be the cause about 80% of fish mortalities ***(Austin and Austin 1987).*** *Aeromonas hydrophila* is known to be one of the most important bacteria associated with diseases among freshwater fishes. The diseases caused by *Aeromonas hydrophila* ranged from acute rapidly fatal septicemia to latent infections and has been referred as hemorrhagic septicemia or aeromonas septicemia.

In aquaculture, traditional methods for treating infective pathogens include a limited number of governmentally approved antibiotics and chemotherapeutics.

However, the disadvantages such as marginal effectiveness and high cost are obvious ***(Sealey and Gatlin, 2001).*** These treatments also may cause the accumulation of chemicals in the environment and/or fish, thus posing potential threats to consumers and the environment.

A group of biological and synthetic compounds that enhance the non specific cellular and humoral defense mechanisms in mammals, such as yeast, vitamin combinations, levamisole, β-glucan, peptidoglycan, chitin and chitosan as well as various products derived from plants and animals are effective in preventing disease ***(Villamil et al., 2003).***

Sanguinarine has been incorporated into swine, bovine, and poultry diets to decrease amino acid degradation, increase feed intake, and promote growth. ***(Tschirner 2004).*** ***Nayar et al., (1998)*** said that Brewer's yeast; S. cerevisiae has been recognized to have potential as a substitute for live food in the production of certain fish or as a potential replacement for fish meal.

MATERIAL AND METHODS

1) Feed additives:

a) Sanguinarine (Sangrovit®):

Sanguinarine (quaternary benzo [c] phenanthridine alkaloid) is the active ingredient of Sangrovit® ***(Tanaka et al., 1993).*** Sangrovit® is supplied in a powder form contains 1.5% Sanguinarine. **Recommended dose in fish:** 50 gm/ton ration (50 ppm Sangrovit®) (50 mg/Kg).

It was obtained from the **Phytobiotics Co, Germany.**

b) Vitamin AD_3E (Baytara®):- Recommended dose: 2 ml/Kg ration. It was obtained from the Delta Vet Center Co. Fish, Common carp (*Cyprinus carpio*) fish were purchased from central Laboratory for Aquaculture Research, Suez Canal University. Fish were transported alive in air pumped large clear polyethylene bags in dechlorinated, tap water that was kept overnight.

Fish were kept in prepared full glass aquaria (100 x 40x 50 cm), these aquaria were used for holding the experimental fish through the period of study and supplied with chlorine free tap water according to ***Innes, (1966),***

electric air pumping machines and filters were conducted to each glass aquarium. Thermostatic heater was used along the course of the study to keep the water temperature 23 ± 1°C. Fish were acclimatized to water environment and kept under observation for 2 weeks before the start of the experiment.

Fish were fed a commercial diet; contains 30 % crude protein. The diet in pellets form and was provided at 3 % of the body weight as described by ***Eurell et al., (1978).***

2) Experimental design:

Fishes (40±2) were divided into four groups each group contained duplicate sets of 25 fish and acclimated for two weeks in the glass aquarium.

The first groups received basal diet free from any additives or treatment. The 2nd, 3rd, and 4th groups fed on ration containing 50 mg/Kg ration, 2 ml/Kg ration and 50 mg/Kg + 2 ml/Kg ration Sanguinarine, Vitamin AD_3E and its combination, respectively for 45 days.

All treatments were mixed with the basal diet and egg as a top dressing and a coating agent to prevent leaching of drug. The pellets were prepared weekly, air dried at room temperature and stored in a refrigerator (4^0C) for daily use.

a) Assessment of growth performance parameters:

Body weight gain (WG), Average daily gain (ADG), feed intake and feed conversion ratio (FCR) were determined at 15, 30, 45 days from beginning of experiment according to ***Ricker (1979).***

b) Haematological and biochemical examination:

Blood samples were collected on days 15, 30, 45 from beginning of experiment. Five fishes were randomly collected from each treatment groups and control group, blood samples were taken by syringes from caudal vessels according to ***Rowley, (1990)*** and divided into 2 parts, the 1st part of blood sample was heparinized for the determineation of some haematological parameters, 2nd part was used for serum collection.

Erythrocytic and total leucocytes (WBCs) **counts** were performed using haemocytometer ***(Miller and Seward, 1971).*** Differential leucocytic counts (DLC) were calculated, estimation of blood haemoglobin and Packed cell volume (PCV %) was done according to ***Schalm, (1986).***

Serum aspartate aminotransferase (sAST) and alanine amino transferase (sALT) activities were determined according to ***Bergermeyer et al., (1986).***

Serum total proteins, albumin and globulin were determined according to ***Henry, (1964).*** Serum urea was determined ***(Numann et al., 1977)*** **and** Serum creatinine **was perfo-**

rmed as described by *Faulkner and King 197).*

e) Bacterial count:

Fish samples were collected at days 15, 30, 45 for bacteriological examination under complete aseptic conditions. One gram of intestine was grind with 9 ml peptone then ten fold serial dilutions was carried out. Each dilution was inoculated in three Petri dishes; from which two were assigned using plate count agar for total bacterial count and the other plate was assigned using MacConky agar for coliform count. ***(Shalaby, et al., 2006).***

Counting of colonies was made for the plates showing 30 -300 colonies per plates.

d) Challenge experiment:

Ten fish from each treated group were injected I/P with 0.2ml /24 hrs broth culture of *Aeromonas hydrophila* strain containing 3 x 10^7 CFU/ml^{-1} on days 45 ***(Gopalakannan and Arul, 2006).***

20 fish from control group were collected, 10 fish were injected I/P ***(Schaperclaus et al., 1992)*** with A.hydrophila strain, the other 10 fish were injected I/P with 0.2 ml phosphate buffered saline (PBS). Clinical signs and Postmortem findings were monitored and recor-ded.

E) Histopathological examinations:-

Tissue specimens from kidney, spleen, liver and intestine of treated and controlled fish were fixed in 10% formalin-saline solution. The tissue microscopic slides were stained with hematoxylin and eosin and examined microscopically ***(Drury and Wallington, 1980).***

h) Data analysis: Statistics were calculated with SPSS for windows version 16.0; the mean values obtained in the different groups were compared by One Way ANOVA.

RESULTS and DISCUSSION:

I) Assessment of growth performance parameters:

The results showed that during the period of experiment, using these feed additives showed significant increase in growth performance (table 1,2).

Table (1): Effects of Sanguinarine (50 g/ton ration), vitamins AD_3E (2 ml/Kg ration) and their combination on Average daily gain (ADG), and weight gain (WG) (Means±S.E.).

Time / Groups	At 15 days		At 30 days		At 45 days	
	ADG	WG	ADG	WG	ADG	WG
Control	0.49^{a}±0.006	7.32^{a}±0.09	0.42^{c}±0.006	6.36^{c}±0.09	0.52^{c}±0.014	7.82^{c}±0.22
Sanguinarine	0.50^{a}±0.043	7.57^{a}±0.65	0.48^{bc}±0.014	7.24^{bc}±0.22	0.71^{ab}±0.010	10.67^{ab}±0.16
Vitamins AD_3E	0.59^{a}±0.03	8.85^{a}±0.50	0.59^{ab}±0.05	8.90^{ab}±0.86	0.76^{ab}±0.08	11.36^{ab}±1.28
Sanguinarine + AD_3E	0.53^{a}±0.060	7.96^{a}±0.72	0.68^{a}±0.066	10.23^{a}±0.54	0.88^{a}±0.086	13.19^{a}±0.34

Means within the same row having different letters (a,b,.) are significantly different at P≤ .05.

These result coordinated with those reported by **Vieira *et al.*, (2008)** who reported that cumulative feed conversion was better in birds fed Sangrovit® alone or in combination with organic acids and with **Rawling *et al.*, (2009)** who found that Sangrovit® had a positive effect on tilapia growth performance.

Table (2): Effects of Sanguinarine (50 g/ton ration), vitamins AD_3E (2 ml/Kg ration) and their combination on feed intake and Feed conversion ratio (FCR) (Means±S.E.) N=5.

Time / Groups	At 15 days		At 30 days		At 45 days	
	Feed intake	FCR	Feed intake	FCR	Feed intake	FCR
Control	1.052^{ab}±0.01	2.16^{a}±0.03	1.272^{a}±0.01	3.00^{a}±0.05	1.463^{b}±0.01	2.81^{a}±0.10
Sanguinarine	1.044^{b}±0.01	2.10^{a}±0.17	1.271^{a}±0.02	2.63^{ab}±0.03	1.488^{ab}±0.03	2.09^{b}±0.07
Vitamins AD_3E	1.072^{a}±0.01	1.83^{a}±0.10	1.338^{a}±0.02	2.29^{bc}±0.20	1.604^{a}±0.04	2.16^{b}±0.20
Sanguinarine + AD_3E	1.060^{ab}±0.01	2.06^{a}±0.27	1.299^{a}±0.02	1.93^{c}±0.15	1.606^{a}±0.05	1.85^{b}±0.13

Means within the same row having different letters (a,b,.) are significantly different at P≤ .05.

Sanguinarine in combination with vitamin AD_3E gave better result than Sanguinarine alone. The final body weight of group which fed diet containing vitamins AD_3E reported high feed intake, weight gain more than control group.

Also these results are in agreement with ***Suhenda and Djajadiredja, (1985)*** who showed that fish like other animals require a dietary source of vit-amin A for proper growth.

The present results are compatible with those reported by ***Sau et al., (2004)*** who found that vitamin (E) has a direct effect on growth performance.

II) Effects of Sanguinarine, vitamins AD_3E and their combinations on haematological and biochemical parameters:

We found non to significant changes in all group, Sanguinarine and Sanguinarine plus vitamins AD_3E were treatments were manifested by clear significant changes when compare with control group (Table 3).

All treated groups showed non significant changes in total leucocytic count in first 15 days but significant increase was observed at 30 and 45 days especially in Sanguinarine plus vitamins AD_3E (9.33, 8.67) when compared to control group.

The result is supported by ***Garcia et al., (2007)*** who found that supplementation with vitamins E is essential for the protection of erythrocytes. So there is a relation between vitamin E supplementation and hematocrit increase in fish ***(Menezes et al., 2006).*** In our opinion the increase in haemogram in fish might be due to the nutritive value of these substances.

Table (3): Effects of Sanguinarine(50 g/ton ration), vitamins AD_3E (2 ml/Kg ration) and their combination on erythrogram (Means±S.E.) N=5.

Time / Group	At 15 days			At 30 days			At 45 days		
	RBCs $\times10^6\mu L$	Hb (g/dL)	PCV %	RBCs $\times10^6\mu L$	Hb (g/dL)	PCV %	RBCs $\times10^6\mu L$	Hb (g/dL)	PCV %
Control	1.54^{bc} ±0.01	8.59^{bc} ±0.3	24.67^{b} ±1.2	1.83^{a} ±0.08	9.67^{a} ±0.2	32.33^{a} ±0.3	1.80^{a} ±0.27	8.50^{bc} ±0.5	27.33^{b} ±0.7
Sanguinarine	1.66^{ab} ±0.07	9.41^{a} ±0.3	29.67^{a} ±1.5	2.01^{a} ±0.21	10.00^{a} ±0.9	33.00^{ab} ±1.2	2.07^{a} ±0.08	9.77^{ab} ±0.7	31.67^{ab} ±1.7
Vitamins AD_3E	1.47^{c} ±0.00	7.85^{c} ±0.3	27.00^{ab} ±0.6	2.13^{a} ±0.20	8.95^{ab} ±1.4	29.33^{b} ±1.8	1.88^{a} ±0.34	8.82^{b} ^{c}±1.1	27.67^{b} ±2.7
Sanguinarine + AD_3E	1.54^{bc} ±0.03	8.52^{bc} ±0.0	26.33^{b} ±0.3	2.40^{a} ±0.48	10.63^{a} ±1.6	34.33^{a} ±0.9	2.24^{a} ±0.38	10.50^{a} ±0.9	33.67^{a} ±2.3

Means within the same row having different letters (a,b,.) are significantly different at P≤.05>

Total leucocytic count of common carp fed on diet containing Sanguinarine, vitamins AD_3E and their combination was illustrated in tables (4a, 4b). It has been shown from this table that: All treated groups showed non significant changes in total leucocytic count except Sanguinarin plus vitamins, meanwhile there was mild changes in all groups in lymphocytic and monocytic count; finally non significant changes in all groups in eosinophiles and basophiles count.

All treated groups showed non significant changes in total leucocytic count at first 15 days but significant increase was observed at 30 days in group treated with Sanguinarine plus vitamins AD_3E ($9.33\times10^3/\mu L$) when compared to the control group $5.67\times10^3/\mu L$ and at 45 days ($8.67\times10^3/\mu L$) when compared to the control group $4.33\times10^3/\mu L$.

All groups revealed increase in erythrocytic count, hemoglobin content and PCV%. In our opinion the increase in haemogram in fish might be due to the nutritive value of these substances.

These results also agree with ***Rawling et al., (2009)*** who reported that total leukocyte levels were elevated in fish fed Sangrovit® supplemented diets, but on the other hand these results were not in accordance with the same authers where they reported that haematological and immunological parameters remained unaffected.

All treated groups showed mild significant changes in biochemical parameters when compared to the control group (Table 5 a, 5 b).

Table (4 a): Effects of Sanguinarin(50 g/ton ration), vitamins AD_3E (2 ml/Kg ration) and their combination on Total leucogram count $\times 10^3$ / µL (Means±S.E.) N=5.

time / Group	At 15 days (Relative %)			At 30 days (Relative %)			AT 45 days (Relative %)		
	T.L.C $\times 10^3$/ µL	Lymphocyte count$\times 10^3$/ µL	Neutrophiles count 10^3/ µL	T.L.C $\times 10^3$/ µL	Lymphocyte count$\times 10^3$/ µL	Neutrophiles count 10^3/ µL	T.L.C $\times 10^3$/ µL	Lymphocyte count$\times 10^3$/ µL	Neutrophiles count 10^3/ µL
Control	3.67^{a} ±0.88	2.59^{a} ±0.66 (69.8)	0.58^{a} ±0.13 (16.9)	5.67 ±0.88	2.84^{b} ±0.40 (50.7)	2.04^{bc} ±0.48 (35.0)	4.33^{b} ±0.33	2.20^{a} ±0.28 (50.3)	1.38^{a} ±0.08 (32.3)
Sanguinarine	4.33^{a} ±0.33	2.89^{a} ±0.21 (66.9)	0.69^{a} ±0.16 (15.8)	7.00^{bc} ±0.58	3.53^{ab} ±0.34 (50.3)	2.06^{bc} ±0.25 (29.3)	7.00^{a} ±0.58	3.76^{a} ±0.36 (54.1)	2.07^{a} ±0.36 (29.2)
Vitamins AD_3E	3.67^{a} ±0.33	2.69^{a} ±0.21 (73.5)	0.34^{a} ±0.07 (9.2)	5.67^{c} ±0.33	2.87^{b} ±0.23 (50.7)	2.04^{bc} ±0.18 (36.0)	6.00^{a} ±1.29	3.21^{a} ±0.96 (54.7)	1.77^{a} ±0.57 (29.7)
Sanguinarine + AD_3E	4.00^{a} ±0.58	2.76^{a} ±0.48 (69.2)	0.65^{a} ±0.26 (16.2)	9.33^{a} ±0.33	4.44^{a} ±0.30 (47.5)	3.23^{a} ±0.21 (34.9)	8.67^{a} ±1.40	4.16^{a} ±1.05 (49.4)	3.13^{a} ±0.05 (34.3)

Means within the same row having different letters (a,b,.) are significantly different at P≤ .05

Table (4 b): Effects of Sanguinarin(50 g/ton ration), vitamins AD_3E (2 ml/Kg ration) and their combination on Total leucogram count $\times 10^3$ / µL (Means±S.E.) N=5.

Time / Group	At 15 days (Relative %)			At 30 days (Relative %)			AT 45 days (Relative %)		
	Monocyte count $\times 10^3$/ µL	eosinophiles count $\times 10^3$/ µL	Basophiles count $\times 10^3$/ µL	Monocyte count $\times 10^3$/ µL	eosinophiles count $\times 10^3$/ µL	Basophiles count $\times 10^3$/ µL	Monocyte count $\times 10^3$/ µL	eosinophiles count $\times 10^3$/ µL	Basophiles count $\times 10^3$/ µL
Control	0.35^{a} ±0.08 (9.7)	0.073^{ab} ±0.02 (2.0)	0.07^{a} ±0.014 (1.7)	0.64^{b} ±0.13 (11.7)	0.073^{a} ±0.02 (1.3)	0.08^{a} ±0.013 (1.3)	0.66^{a} ±0.14 (15.0)	0.043^{a} ±0.00 (1.0)	0.06^{a} ±0.012 (1.3)
Sanguinarine	0.61^{a} ±0.01 (14.3)	0.103^{ab} ±0.02 (2.3)	0.03^{a} ±0.015 (0.7)	1.26^{a} ±0.09 (18.0)	0.090^{a} ±0.02 (1.3)	0.07^{a} ±0.021 (1.0)	0.99^{a} ±0.13 (14.0)	0.107^{a} ±0.05 (1.7)	0.08^{a} ±0.026 (1.0)
Vitamins AD_3E	0.55^{a} ±0.10 (14.7)	0.070^{ab} ±0.04 (2.0)	0.02^{a} ±0.012 (0.7)	0.60^{b} ±0.03 (10.7)	0.093^{a} ±0.02 (1.7)	0.06^{a} ±0.020 (1.0)	0.84^{a} ±0.38 (13.0)	0.093^{a} ±0.05 (1.3)	0.09^{a} ±0.023 (1.3)

Sanguinarine + AD_3E	0.48 [a] ±0.09 (12.0)	0.063[b] ±0.01 (1.7)	0.04[a] ±0.006 (1.0)	1.44[a] ±0.13 (15.3)	0.160[a] ±0.04 (1.7)	0.06[a] ±0.023 (0.7)	1.23 [a] ±0.36 (14.7)	0.133[a] ±0.011 (1.3)	0.01[a] ±0.013 (0.3)

Means within the same row having different letters (a,b,.) are significantly different at P≤ .05

Comparing to the total serum globulin of the control group it was cleared that there was significant increasing in the total serum globulin of the all treated groups, but it was highly significant in group treated with Sanguinarine plus vitamins AD_3E.

The results are compatible with those reported by ***Rawling et al., (2009)*** who reported that hepatic alanine aminotransferase activity remained unaffected in fish fed Sangrovit® diets.

Table (5 a): Effects of Sanguinarin(50 g/ton ration), vitamins AD3E (2 ml/Kg ration) and their combination on on biochemical parameters. Means±S.E.) N=5.

Time	At 15 days			At 30 days			At 45 days		
Group	ALT (U/ml)	AST (U/ml)	T.Protein g/dL	ALT (U/ml)	AST (U/ml)	T.Protein g/dL	ALT (U/ml)	AST (U/ml)	T.Protein g/dL
Control	34.43[a] ±0.44	59.12[b] ±0.32	4.690b[c] ±0.06	32.67[a] ±1.84	61.95[a] ±0.69	4.425[abc] ±0.28	34.14[a] ±0.76	59.35[b] ±1.73	3.974[b] ±0.08
Sanguinarine	31.02[a] ±0.70	56.00[d] ±0.13	4.427[d] ±0.04	33.29[a] ±1.06	62.00[a] ±1.15	3.796[c] ±0.11	32.47[a] ±0.74	58.50[bc] ±0.26	4.910[a] ±0.42
Vitamins AD_3E	31.79[a] ±1.05	56.43[cd] ±0.53	3.943[e] ±0.09	29.80[a] ±0.65	57.93[b] ±0.53	4.850[a] ±0.49	30.74[a] ±1.37	57.10[bc] ±0.38	4.023[b] ±0.12
Sanguinarine + AD_3E	31.16[a] ±1.48	57.18[c] ±0.41	4.446[cd] ±0.14	32.34[a] ±0.50	60.63[ab] ±0.81	4.678[ab] ±0.15	31.56[a] ±1.31	55.99[c] ±0.87	5.286[a] ±0.25

Means within the same row having different letters (a,b,.) are significantly different at P≤ .05.

Table (5 b): Effects of Sanguinarin(50 g/ton ration), vitamins AD3E (2 ml/Kg ration) and their combination on on biochemical parameters. Means±S.E.) N=5.

Time / Group	At 15 days			At 30 days			At 45 days		
	Albumin g/dL	Urea mg/ dl.	Creatinine mg/ dl.	Albumin g/dL	Urea mg/ dl.	Creatinine mg/ dl.	Albumin g/dL	Urea mg/ dl.	Creatinine mg/ dl.
Control	1.824^{a} ±0.042	5.08^{ab} ±0.15	0.703^{a} ±0.034	1.533^{c} ±0.048	5.30^{ab} ±0.13	0.720^{a} ±0.012	1.434^{c} ±0.009	4.96^{a} ±0.17	0.760^{a} ±0.021
Sanguinarine	1.361^{d} ±0.036	4.74^{b} ±0.04	0.713^{a} ±0.018	1.427^{d} ±0.019	5.52^{ab} ±0.39	0.703^{a} ±0.003	1.495^{c} ±0.020	5.03^{a} ±0.24	0.683^{b} ±0.009
Vitamins AD_3E	1.697^{ab} ±0.006	4.99^{ab} ±0.12	0.717^{a} ±0.015	1.916^{a} ±0.031	5.69^{ab} ±0.35	0.667^{a} ±0.027	2.074^{a} ±0.054	5.18^{a} ±0.30	0.687^{b} ±0.009
Sanguinarine + AD_3E	1.282^{cd} ±0.158	4.84^{ab} ±0.25	0.713^{a} ±0.018	1.555^{c} ±0.014	6.09^{a} ±0.01	0.687^{a} ±0.026	1.517^{c} ±0.007	5.51^{a} ±0.30	0.670^{b} ±0.015

Means within the same row having different letters (a,b,.) are significantly different at P≤ .05

III) Effects of Sanguinarine, vitamins AD_3E and their combinations on total bacterial count:

Group treated with Sanguinarine plus vitamins AD_3E showed highly significant decrease in total bacterial count 43.44×10^{6} CFU followed by Sanguinarine, and finally vitamins AD_3E 53.10, 63.49, 66.11, 79.44×10^{6} CFU respectively when compared to the control group 83.33×10^{6} CFU (Table 6).

Group treated with Sanguinarine showed highly significant decrease in total coliform count 7.43×10^{5} CFU followed by Sanguinarine plus vitamins AD_3E, finally vitamins AD_3E 7.83, 9.49, 9.73, 11.53×10^{5} CFU respectively when compared to the control group 16.44×10^{5} CFU.

The obtained results are compatible with those reported by ***Dzink and Socransky, (1985)*** who stated that Quaternary benzo[c] phenanthridine alkaloids have antimicrobial activity at minimum inhibitory concentrations for several bacteria.

Many of these bacteria belong to the genus commonly found in the gastrointestinal tract.

Table (6): Effects of Sanguinarin (50 g/ton ration) vitamins AD_3E (2 ml/Kg ration) and their combination on total bacterial count (T.B.C) and coliform count (CFU)

Time / Groups	At 15 days		At 30 days		At 45 days	
	T.B.C $\times10^6$	Coliform count $\times 10^5$	T.B.C $\times10^6$	coliform $\times10^5$	T.B.C $\times10^6$	coliform count $\times10^5$
Control	40.10^a ±0.06	7.00^a ±0.06	92.57^b ±0.07	15.50^{ad} ±0.26	117.33^a ±1.45	26.83^a ±0.44
Sanguinarine	29.10^c ±0.06	0.85^e ±0.03	62.10^c ±0.06	4.60^f ±0.06	68.10^d ±0.06	16.83^a ±0.12
Vitamins AD_3E	37.10^b ±0.06	4.83^b ±0.33	100.23^a ±0.12	8.93^b ±0.03	101.00^b ±0.58	20.83^a ±0.44
Sanguinarine + AD_3E	25.10^f ±0.06	2.27^d ±0.12	53.10^f ±0.06	6.70^e ±0.06	52.13^e ±0.09	14.53^a ±0.09

Means within the same row having different letters (a,b,.) are significantly different at P≤.05.

These results were supported by ***Newton et al; (2002)*** who found that natural compounds extracted from plants, such as the quaternary benzo [c] phenanthridine alkaloids (**QBA**) sanguinarine and chelerythrine, are known to have antimicrobial effect.

V) Clinical signs and postmortem lesions due to challenge test:-

The clinical signs appear in ***Cyprinus carpio*** in response to I/P injection of ***Aeromonas hydrophila*** were similar but varied in the severity of developed lesions.

Darkening of the body color (**Fig.1**), with large red irregular hemorrhages on the body surface.

Also presence of congestion and hemorrhage of all fins.

Abdominal distension due to accumulation of ascetic fluid in abdominal cavity with protruded anal opening (**Fig.2**). The epidermis was eroded leaving skeletal muscle exposed (loosed leaving ulcers) (**Fig .3**), finally loss of all reflexes just prior

to death.

Internally, congestion of all internal organs with yellowish serous fluid in the abdominal cavity **(Fig.4)**, was found enlarged liver with hemorrhagic patches, inflamed foci, neorotic areas and distended gallbladder with bile **(Fig.5)**. The intestine may be inflamed and hyperaemic containing yellowish mucus and was voided of food **(Fig.6)**.

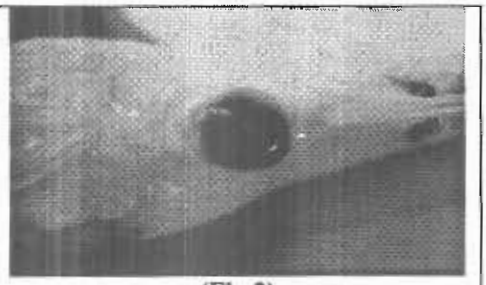
Kidney appeared swollen and congested.

These results were confirmed by ***West et al., (1991)*** who found Vitamin A has a significant effects on innate and specific immune responses.

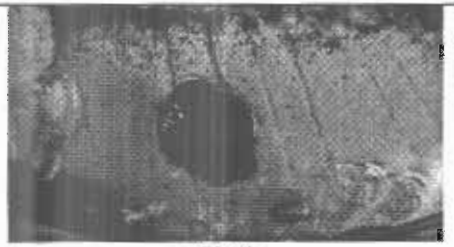
Rebeca et al., (2009) suggested that dietary vitamin D_3 administration has an effect on the innate immune parameters of gilthead seabream. The immunostimulant effect was greater on the cellular innate immune parameters.

The obtained results are compatible with those reported by ***Montero et al., (1999)*** who said that Vitamin E provides additional health protection through its immunostimulant property, and it seemed to have more protective role against stress.

(Fig.1)



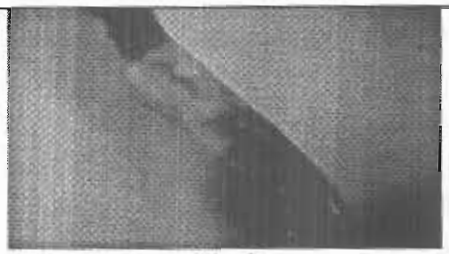
(Fig.2)



(Fig.3)

(Fig.4)

(Fig.5)



(Fig.6)

V) Histopathological finding:-

a) Non treated fish (control group):

The examined organs of control groups (kidney, liver, spleen, intestine) showed no pathological alteration where the cellular details and tissue architecture where normal with no marked changes in the hematpieotic organ along the 45days.

b) Fish treated by Sanguinarine:

The kidney:

The kidney showed focal areas of the hematopoietic tissues were hyperplastic at 15 day. By 30 and 45 days; it displayed hyperplasia of the hematopoietic tissue and melanomacrophages.

The spleen: by 30 and 45 days; it showed hyperplasia of lymphoid follicle (Fig. 1).

d) Fish treated by vitamins AD_3E:

The kidney:

At 15 days it showed swelling of renal tubules with no alteration evident in the hematopoietic tissue. By 30 and 45 days found hyperplasia of the hematopoietic tissue.

The spleen: It revealed edema, mild activation of melanomacrophages and hyperplasia in the lymphoid follicle at 15 days (Fig. 2). By 30 and 45 days it displayed marked lymphoid hyperplasia and focal activation of melanomacrophages. (Fig.3).

e) Fish treated by Sanguinarine plus vitamins AD_3E:

The kidney: The kidney of common carp at 15 day of experiment displayed edema and hyperplasia of the hematopoietic tissue (Fig. 4). At 30 and 45 days it displayed activation of melanomacrophages and hematopoietic tissue with mild degeneration of renal tubules.

The liver: It showed early proliferation of macrophage.

The spleen: It revealed congestion, mild activation of melanomacrophages and proliferation of lymphocyte.

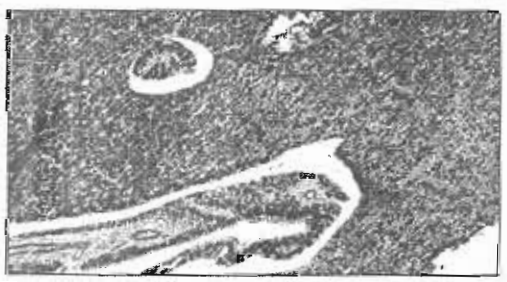


Fig (1)

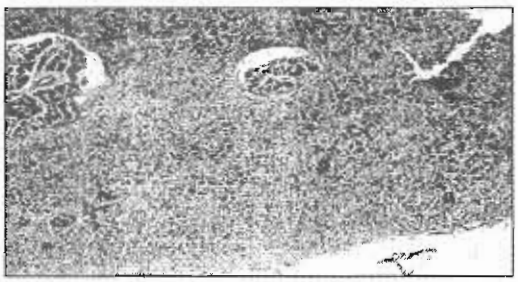


Fig (2)

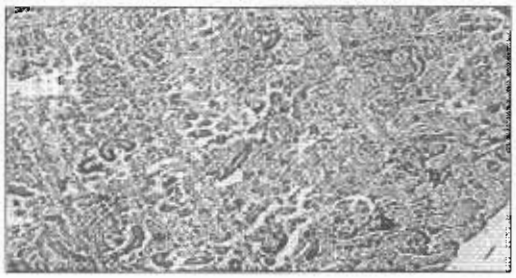


Fig (3)

Fig (4)

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الملخص العربى

تأثير مادة السانجونارين وفيتامينات أدوهـ على معدلات النمو فى سمك المبروك

حـاتم عبد الخالق جمـاز- مصطفى فايز محمد حسن

إيمان على عبد النبى أحمد

كلية الطب البيطرى – جامعة قناة السويس- الاسماعيلية – مصر

الهدف من هذه الدراسة هو تقييم تأثير بعض اضافات الأعلاف وهذة الأضافات هى أضافات نباتية (سانجروفيت) و الفيتامينات على اداء ومؤشرات النمو لاسماك المبروك، وصورة الدم وبعض الاختبارات الكيميانية الخاصة بوظائف الكبد والكلى ، العد البكتيرى والكوليفورم، الاستجابة المناعية بعد تعرضها للاصابة بالمرض بالإضافة الى عمل الفحص الهستوباثولوجى لبعض انسجة الجسم. قسمت الاسماك الى اربعه مجموعات كل مجموعة تحتوى على 25 سمكة تمت معاملتها معاملة واحدة من حيث المناخ والرطوبة والاضاءة وقد غذت الاسماك على عليقة بمعدل 3% من وزن السمكة. .

وكانت المجموعات على النحو التالى:-

المجموعة الاولى مجموعة ضابطة تناولت عليقة بدون اضافات. المجموعة الثانية و الثالثة و الرابعة تناولت عليقة مضافا اليها السانجروفيت بمعدل 50 جزء فى المليون و فيتامينات بمعدل 2 ملل/كجم والسانجروفيت 50 جزء فى المليون + فيتامينات 2 ملى/كجم على التوالى طول فترة التجربة.

وقد تم وزن كل المجاميع فى بداية التجربة ثم فى نهاية كل 15 يوم مع حساب معدل الزيادة فى الاوزان ومعدل التحول الغذائى. تم تجميع عينات الدم لعمل صورة دم كاملة وعمل تحاليل خاصة بوظائف الكبد والكلى عند 15-30-45 يوم وتم اجراء العد البكتيري وعد بكتيريا الكوليفورم فى الامعاء بالاضافة الى عمل الفحص الهيستوباثولوجى لبعض انسجة الجسم (الكبد-الكلى-الطحال-الامعاء).

وتم حقن الاسماك بميكروب الايروموناس هيدروفيلا فى كل المعاملات لمعرفة مدى تأثير هذة الاضافات على مدى مقاومة الاصابة بهذا الميكروب.

وقد اظهرت النتائج مايلى: اسفرت النتائج عن زيادة فى اوزان جميع المجموعات مع زيادة المتوسط اليومى للاسماك وزيادة الكفائة الغذائية فى كل المجاميع فيما عدا المجموعة الثانية المعالجة بالسانجروفيت حيث لم يتأثر استهلاكها للعليقة مقارنة بالمجموعة الضابطة. وكانت المجموعة الثانية المعالجة بالسانجروفيت منفردا و المجموعة المعالجة بالسانجروفيت + الفيتامينات الافضل من حيث صورة الدم حيث وجد تحسن فى عدد خلايا الدم الحمراء ونسبة الهيموجلوبين مع زيادة فى عدد خلايا الدم البيضاء. كما اظهرت النتائج ان السانجروفيت لة تأثير قوى كقاتل للبكتيريا المعوية ككل وخاصة الكوليفورم كما اظهرت المجاميع الاخرى نقص فى العدد البكتيرى ايضا قد يعزى الى الزيادة المناعية.

وقد كانت نسبة ظهور الأعراض فى أسماك المجموعة الضابطة اعلى ما يمكن وقد اعطت صورة واضحة للمرض وظهرت هذه الاعراض ايضا فى باقي المجموعات ولكن بصورة اقل وقد صاحبها تغيرات هستوباثولوجية كانت اكثر وضوحا فى المجموعة الضابطة.لذلك توصى الدراسة بأستخدام الاضافات العلفية (المستخلصات النباتية و الفيتامينات) الى الاسماك المستزرعة.