Assiut Vet. Med. J. Vol. 41 No. 82, July 1999.

Dept.of Animal Hygiene
Fac. of Vet.Med., Assiut Univ.
Head of Dept.:Prof.Dr.A.H.Hafez

EFFECT OF ASCORBIC ACID SUPPLEMENTATION ON THE GROWTH PERFORMANCE AND PATHOLOGY OF TILAPIA FISH SUBJECTED TO BACTERIAL INFECTION (*Pseudomonas fluorescens*)

(With 12 Figures and 8 Tables)

By

A. N. SAYED and S. Kh. ABD-ELGHAFFAR*

*Dept.of pathology & clinical pathology, Fac.of Vet.Med., Assiut Univ.

(Received at 23/6/1999)

مدى تأثير حمض الأسكوربيك على أداء وباثولوجيا أسماك البلطي المعرضة للعدوى البكتيرية (ميكروب السيدومونس فلوريسنس)

عبد الباسط نصر سيد ، ساري خليل عبد الغفار

في هذه الدراسة تم أجراء تجربتين لتقييم مدى تأثير حمض الأسكوربيك علـــى أداء ومعـــدلات النمو في سمك البلطي في التجربة الأولى ومدى مقاومة هـــذه الأســماك للعـــدوى البكتيريـــة بميكروب السيدومونس في التجربة الثانية. تم تغذية الأسماك التي تزن ٢٠ جم علـــى علائـــق تحتوي علي مستويات مختلفة من حمض الأسكوربيك (صفـــر, ٥٠, ١٥٠, ٢٥٠, ٥٠٠, ١٠٠٠ مجم/كجم عليقة) لمدة ١٢ أسبوع في كل تجربة. التجربة الأولى:- وجد أن معدلات النمو كـــانت أقل معنوياً في مجموعة الأسماك التي غذيت علي العليقـــة التـــي لا تحتـــوي علـــي حمـــض الأسكوربيك مقارنة بالمجموعات الأخرى التي غذيت على العلائق التي تحتوي علـــى حمـــض الأسكوربيك. كذلك وجد أن ٥٠ مجم من حمض الأسكوربيك كافية لمنع ظـــهور أي أعـــراض مرضية على الأسماك بينما مستوى ١٥٠ مجم حمض الأسكوربيك أدى إلـــى حـــدوث تحســـن معنوي في كل من وزن الجسم النهائي ومعدل التحويل الغذائي والكفاءة التحويليـــة للـــبروتين. زيادة حمض الأسكوربيك في العلائق أدى إلى زيادة معنوية في محتوى جســـم الأســـماك مـــن البروتين وانخفاض في مستوى الدهون. كذلك وجد أن أعلى نسبة نفوق كانت فـــي مجموعـــة الأسماك التي غذيت على العليقة التي لا تحتوي على حمض الأسكوربيك مع وجود نزيف دموي على الفم وكذلك وجود بعض التشوهات الخلقية في جسم الأسماك. وجود زيـــادة معنويـــة فـــي محتوى أنسجة جسم الأسماك من حمض الأسكوربيك مع زيادة مستوى حمض الأسكوربيك فـــي العلائق وأن أعلى تركيز سجل في المجموعة التي غذيت على العليقة التي تحتوي علـــى ٥٠٠ مجم حمض الأسكوربيك في الطحال والكبد والعضلات والكلى على التوالى. التجربة الثانيـــة:- غذيت مجموعات الأسماك على علائق تحتوي على مستويات مختلفة من حمض الأســـكوربيك (صفر، ٥٠، ١٥٠، ٢٥٠، ٥٠٠، ١٠٠٠ مجم/ كجم عليقة) حتـــى ظـــهور أعـــراض النقـــص

المرضية على مجموعة الأسماك التي غذيت على العليقة التي لا تحتوي على حمض الأسكوربيك بعد ذلك تم عدوى كل المجموعات ببكتريا مرضية تم عزلها من الأسماك (ميكروب السيدومونس فلوريسنس) وقد وجد أن:- أعلى نسبة نفوق كانت في مجموعة الأسماك التي غذيت على العليقة التي لا تحتوي على حمض الأسكوربيك (٩٠%) وقلت هذه النسبة مع زيادة مستوى حمض الأسكوربيك في العلائق حتى ١٠٠٠ مجم (١٠%). وقد لوحظ أن صورة الدم قد تحسنت والآفات الباثولوجية قد قلت تدريجياً مع زيادة تركيز حمض الأسكوربيك في العليقة.

SUMMARY

Two experiments were conducted in this study to determine the effect of ascorbic acid level on the growth performance and pathology of tilapia fish subjected to bacterial infection. Purified diets with six levels (0, 50, 150, 250, 500 & 1000 mg/kg) of supplemental L-ascorbic acid (AA) were pelleted and fed to tilapia fish (20 g) for 12 weeks in each experiment. *In the first experiment:-* The growth rate of fish fed AA free diet was significantly ($P<0.05$) lower than those fed on dietary supplemented ascorbic acid. Approximately, 50 mg AA/kg diet was sufficient to prevent the appearance of deficiency symptoms, while a level of 150 mg AA/kg diet significantly ($P<0.05$) improved final body weight, feed conversion, specific growth rate and protein efficiency ratio. Increasing AA level in the diets significantly ($P<0.05$) increase the crude protein content of the body, on the contrary to fat content which decreased by the increase of AA level. Maximum mortality was recorded with fish fed on the AA free diet with haemorrhage at the mouth and deformities. The AA contents of the body tissues increased significantly ($P<0.05$) with increasing AA level up to 500 mg/kg diet. The highest tissue concentration of AA was recorded at the level of 500 mg/ kg diet and storage was found to be maximal in the spleen followed by liver, muscle and kidney. *In the second experiment:-* Tilapia fish were fed purified diets containing AA ranging from 0 (free diet) to 1000 mg/kg until the external signs of deficiency were seen in fish fed AA free diet. At this time, resistance to bacterial infection (*Pseudomonas fluorescens*) was assessed for fish from the various dietary treatments. Mortality rates of fish experimentally infected with *P.fluorescens* decreased with increasing the dietary AA doses, ranging from 90% for fish fed the AA-free diet to 10% for fish fed 1000mg AA/kg diet.The haematological parameters were improved and the pathological lesions of bacterial infection were minimized gradually with increasing the ascorbic acid concentration in the diets.

***Key words*:-** Ascorbic acid, *Pseudomonas fluorescens*, Pathology, Tilapia

INTRODUCTION

Ascorbic acid is an indespensable nutrient required to maintain the physiological processes of different animals including fish (Tolbert, 1979). Fish such as salmon, catfish, trout, carp, tilapia and probably others have not the ability to synthesize ascorbic acid due to absence of the enzyme L-gulanolactone oxidase responsible for synthesis of vit.C (Chatterjee, 1978), but are dependent upon a dietary source of vit.C (Fenster, 1987; Dabrowski, 1990). These kinds of fish were shown to develop specific avitaminosis C symptoms such as lordosis and scoliosis with resultant high mortality. It has been established that supplementing fish feed with vit.C is essential to avoid such lesions, but it was also of benefit in the resistance to bacterial or viral infections (Hornig et al., 1984). Ascorbic acid is essential for maximum rate of immune responses, and has a role in detoxification of various xenobiotics (Lovell, 1989). The requirement of vit.C was based on the promise that the dietary intake sufficient to promote maximum liver tissue storage should satisfy tissue demands of the vitamin for maximum growth, resistance to disease and normal stress encountered in the environment (Halver, 1979). Hilton et al. (1978) found that 20 mg of vit.C/kg diet was sufficient for normal growth in rainbow trout, but 40mg/kg was necessary to prevent gross deficiency signs. Li & Lovell (1984) found similar results with channel catfish. Many abnormalities such as spinal deformities, growth retardation, mouth haemorrhage, gill hyperplasia, anorexia, caudal fin erosion, exophthalmia and high mortality were observed in fish fed diets containing none or only a low dose of vit.C (Soliman et al., 1986 and Grant et al., 1989). Of all essential micronutrients, vit.C has generated the greatest interest with regard to its interactions with defensive mechanisms and the immune system which include antibacterial and antiviral effects (Beisel, 1982). Essential nutrients, such as vitamins (C & E) may affect not only humoral and cell mediated immune response, but also several nonspecific humoral factors, such as lysozymes or hormones which regulate the immune response (Weber, 1997). Vit.C have been shown to affect immunity and disease resistance in salmonids (Paterson et al., 1985; Blazer and Wolke, 1984) as well as channel catfish (Durve and Lovell, 1982; Li and Lovell, 1985). However, relatively little is known about the relationship between vit.C and disease resistance in tilapia fish.

The purpose of this study was to determine the relationships between dietary levels of vit.C and growth performance, tissue storage of vit.C, pathology and disease resistance to bacterial infections in tilapia fish.

MATERIALS and METHODS

A- Fish and management:-

Tilapia fish fingerlings (*O.niloticus*) were obtained from Aquatic Animal Research Unit ,Fac.of Vet.Med.,Assiut Univ. ,which supposed to be free from diseases, and with an average initial weight and length 20±0.10g & 10.80±0.10cm, respectively. The fish were divided into equal groups (20 fish per each group) and distributed to the experimental glass aquaria. Each aquarium was aerated and contained dechlorinated tap water. The water temperature, dissolved oxygen and pH of water were measured and found to be 26 °C± 1°C ,3.8mg/L and 7.2 respectively. To minimize stress of handling ,fish from each aquarium were weighed and the total length of each fish was measured at the beginning and end of the study.

B- Ascorbic acid :-

The L-ascorbic acid was purchased from Nasr Chemical Company.

C-Experimental diets:-

All the experimental diets were intended to be isonitrogenous (32.00% protein) and isocaloric (3.14 Kcal/g digestible energy) and were formulated to satisfy the requirement of tilapia fingerlings (NRC, 1993). The chemical composition of the experimental diets are shown in table (1).

Six experimental diets were assigned composed of the basal diet plus 0, 50, 150, 250, 500, 1000mg vit.C/Kg diet. The amount of ascorbic acid (AA) was substituted with the equal amount of wheat bran in the basal diet.

In preparing the diets ,dry ingredients were first ground to small particle size in a mill and were thoroughly mixed ,combined with water in mixer, pelleted by forcing through 4mm holes and dried at room temperature. The pellets were sealed in plastic bags and frozen immediately to minimize oxidative loss of ascorbic acid. The diets were stored at -23°C and periodically one week diet of each was transferred to a refrigerator 4°C and held prior to feeding. Previous analysis showed that there was approximately a 5% loss of ascorbic acid per 10 wk under this storage condition (Lim & Lovell, 1978).

After 15 days adaptation period, fish were fed to satiation on the experimental diets (two meals per day at 08.00 and 17.00 h).

D- Pseudomonas fluorescens isolate:-

A well identified *Pseudomonas fluorescens* bacterial isolate, isolated from naturally infected *O.niloticus* was kindly supplied by the Aquatic

Animal Research Unit. The isolate was propagated in Brain heart infusion agar media to be used in the experimental infections at a final dilution of 172x 10^8 ml (Total bacterial count).

E- Experimental designs:-

Two separate experiments were conducted in this study.

1- First experiment:-

Six groups of fish were used in this experiment (1st, 2nd, 3rd, 4th, 5th, & 6th group). Each group was fed on one of the different experimental diets which contain 0, 50, 150, 250, 500 & 1000 mg ascorbic acid / kg diet respectively. Fish were visually checked periodically for the deficiency symptoms of vit.C and spinal deformities. At the end of the experiment (12 weeks), fish were weighed, feed intake was recorded. Mortality was also recorded from the start of the exp. The fish were then sacrificed for further studies.

2-Second experiment :-

Another six groups of fish were used in this experiment (1st, 2nd, 3rd, 4th, 5th, & 6th group) and fed on the experimental diets as in exp. I.

After 10 weeks of feeding, when clinical signs of deficiency of vit.C were seen in 30% of fish group fed on the vit.C free diet (1st group), fish from each dietary treatment were inoculated intra-peritonealy with 0.05ml of *Pseudomonas* broth culture.

After infection, all fish were returned to rearing aquaria and mortalities were recorded. After 2 weeks of infection, approximately 2ml of blood was drawn from the caudal vein of the fish of each group for determination of some haematological parameters (Coles, 1986). At the end of the experiment (12 weeks), fish were sacrificed and tissue speciemens were taken for histopathology.

F- Analytical techniques:-

Dry matter ,crude protein ,ether extract and ash contents of the fish tissues were performed according to AOAC (1984). Tissue vit.C concentration were measured after Schuep et al. (1984).

G- Pathological examination:-

Speciemens from liver, spleen, testes and intestine were taken from sacrificed fish of the second experiment. The speciemens were fixed in neutral buffer formalin. The fixed samples were dehydrated in alcohols, processed and embedded in paraffin blocks. Sections of 5-7 micron were prepared, stained with haematoxylin and eosin (Bancroft and Stevens, 1993), and then examined under light microscope.

H- Haematological examination:-

Blood samples were collected from the sacrificed fish at the end of the experiment. The samples were used for the detection of :-

1-Total erythrocytic and leucocytic count/mm^3 blood using Haemocytometer.

2-Differential leucocytic count on blood film stained with wrights stain.

I-Statistical analysis:-

Statistical comparisons were made using a one-way analysis of variance (ANOVA). Mean differences between treatments were tested for significance ($P<0.05$) by Duncan's multiple range test (1955). Standard errors ($\pm$SE) were calculated to indicate the range of means tested.

RESULTS

A- First experiment:-

1- Growth performance and tissue vit.C storage:-

All the data of this experiment are summarized in the Tables (2-7).

B-Second experiment :-

1-Gross lesions :-

Small haemorrhagic skin lesions, peritoneal peticheal haemorrhages, enlargement and paleness of the liver and ascitis were seen in some cases in addition to symptoms of vit.C deficiency, especially at the 1st group (0 vit.C level). These lesions and symptoms were minimized in 2nd, 3rd groups and sporadically seen in 5th and 6th groups.

2- Histopathology:-

a-Liver:-

In the 1st group (0 vit.C level), there were diffuse fatty change in the hepatocytes (Fig. 1). This hepatic lesion was minimized in the groups which fed on the diets with high levels of vit. C, while it was focally scattered (Fig.2) in the 2nd and 3rd groups (50, 150 mg vit.C). In the 4th group (250 mg vit.C), there were a mild hydropic degeneration of hepatocytes (Fig. 3). The 5th & 6th groups (500 & 1000 mg vit.C) showed more or less normal histological structure of the liver (Fig. 4).

b-Spleen :-

Exhuastion of lymphocytic elements was the predominent lesions in the spleen, specially in the 1st group. The splenic tissue appeared as a mesh of reticular fibers denuded from lymphocytic elements. The melonocytes in melano-macrophage center were rupture (Fig.5). In the 2nd and 3rd groups,

the exhuastion was minimized (Fig. 6). In the 5th & 6th groups, the spleen appear more or less normal (Fig.7 & 8).

c-Intestine :-

The intestine in the first three groups showed necrosis and desquamation of the epithelium at the tips of the villi (Fig. 9), while this changed was completely disappeared and the intestine appeared normally in the last three groups (Fig. 10).

d-Testes:-

Degeneration and necrobiosis in spermatogenic cells were seen in the semineferous tubules of the testes in the 1st group (Fig., 11). The spermatogenesis were gradually appeared in the groups which fed on high levels of vit.C (Fig. 12).

3- Haematology:-

The haematological parameters were improved by the supplementation of ascorbic acid in the diets. This was manifested by increasing in the number of total erythrocytic and leucocytic count. There was a relative increase in the percentage of lymphocyte and monocyte with decrease in the percentage of heterophil cells in peripheral blood (Table,8).

DISCUSSION

1- The first experiment:-

The signs of ascorbic acid deficiency which showed on the group of fish fed on the ascorbic acid free diet during 12 weeks characterized by scoliosis, lordosis, haemorrhage at the base of mouth, fins, operculum and anus, in addition to anorexia. These symptoms were found in other species of fish as salmon and trout (Halver et al.,1969; Hilton et al.,1978 & Sato et al.,1983), tilapia fish (Halver, 1979; Stickney et al., 1984 & Soliman et al., 1986), channel catfish (Wilson and Poe,1973; Andrew and Murai,1974; Lim and Lovell, 1978), major carp (Agrawal & Mahajan, 1980), common carp (Dabrowski et al.,1988). Scoliosis appeared in fish in 1-3 weeks in tryptophan free diet and in 5-6 weeks in ascorbic acid free diet (Lovell,1984). Halver et al. (1969) showed extensive haemorrhage in conjunction with the distortion in salmon, but in tilapia at tropical temperature where the onset of the clinical signs was earlier, the haemorrhage was not a necessary concomitant (Soliman et al.,1985).

Maximum mortality was observed in the fish group fed on the AA free diet (Table, 2). The observed high mortality rate in fish fed AA free diet agreed with the observations of Navarre& Halver (1989) with rainbow

trout and Al-Amoudi et al. (1992) with tilapia fish. Deformities were identified visually at the end of the first experiment in 50% of the fish fed the AA free diet. Most common symptoms were scoliosis, a lateral curvature of the spinal column. Many fish showed lordosis, with or without scoliosis, which was most commonly characterized by a large hump. Condition factors (C.F) of the fish ranged from 1.81 (250mg AA) to 2.17 (free AA diet). This agreed with that found by Poston (1967) in trout fish, where there was an increase in the condition factor in the fish fed on AA free diet as a result of hump formation.

Body weight of fish fed the ascorbic acid free diet was significantly ($P<0.05$) lower than those of fish fed a dietary supplement of ascorbic acid (Table, 3). The highest growth rate was recorded with fish group fed on diet containing 150mg of AA/Kg. This result came in agreement with that reported by Al-Amoudi et al. (1992) and Jauncey & Ross (1982) for tilapia. There was no significant difference ($P>0.05$) in the body weight or feed conversion between fish groups fed on the diets containing 150 or 1000mg of AA/Kg diet. The improved growth performance due to ascorbic acid may be attributed to improvement of fish viability, optimization of feed conversion and the utilization of nutrients which led to significant higher growth rate as reported by Kitamura et al. (1965). Fenster (1987) reported that AA was found to be involved in biochemical reactions of practically all groups of nutrients and had a sparing effect on various B-vitamins and vit.E, so, sufficient AA in the diet not only improves the viability of young fish but also significantly raises growth rate, optimizes feed conversion and the utilization of nutrients.

This study showed that dietary AA level of 50mg AA/Kg was sufficient to prevent appearance of deficiency symptoms, while 150mg AA/Kg was required for maximum growth in tilapia fish. Similar conclusions have been obtained with rainbow trout (Hilton et al., 1978; Sato et al., 1978), common carp (Ikeda & Sato,1964) and channel catfish (Murai et al., 1978; LI & Lovell, 1985).

Fish group fed on diet deficient in AA showed reduced growth, drop in feed intake and poor feed utilization as found by other fish species; rainbow trout (Hilton et al.,1977), channel catfish (Lovell,1973; Wilson & Poe, 1973; Andrews& Murai, 1974; Miyazaki et al., 1985; Gatlin & Wilson,1986), Indian carp (Mahajan & Agrawal, 1980; Agrawal & Mahajan, 1980). The growth inhibition observed in response to AA deficiency is partially due to anorexia and diminished activity as found by Soliman et al. (1986) with *O.niloticus*.

Dry matter contents of the whole body of fish flactuated irrespective of dietary AA ranging from 27 to 32%. However, protein and ash contents significantly increased ($P<0.05$) and fat content flactuated with the increasing level of AA in the diets as shown in Table (4). High levels of plasma triglycerides and cholesterol was found in salmon fish fed on free dietary AA as reported by John et al. (1979).

The lowest value of protein efficiency ratio (PER), protein productive value (PPV), apparent protein digestibility, dry matter retention and apparent dry matter digestibility were recorded with fish group fed on AA free diet (Table, 5). These parameters were increased to reach its maximum at 1000 mg AA supplementation with the exception of PER which reached its highest level at 150 mg AA supplementation.

Ascorbic acid is widely distributed throughout the tissues, both in animals capable of synthesizing AA as well as in those dependent on an adequate amount of AA in the diet. The accumulation of AA was found to be maximal in the spleen followed by liver, muscle and kidney (Table,6). Spleen had the highest concentration of AA which ranged from 12.5 to 81.4mg/100g. In studies of various Indian carp, the highest level of AA was found in spleen, followed by the kidney, gonads and liver. Liver is the storage organ and its AA concentration was reported to be a good index of the vitamin status in rainbow trout (Hilton et al.,1977; Skelbaek et al., 1990), channel catfish (Lim & Lovell, 1978) and Tilapia (Al-Amoudi et al., 1992). For tilapia, Al-Amoudi et al. (1992) showed the signs of AA deficiency at 10mg/100g liver. The AA contents of muscles have a definite correlation with the dietary AA, but the amount of AA was lower than that in other organs such as spleen and liver. The reason may be the activeness of the muscle; AA in muscle may be in the consumable form, which is readily available for physiological activities as found by Jauncey et al. (1985) and AL-Amoudi et al. (1992). Halver (1989) recommended the use of liver ascorbate concentration as a clinical assessment of vit.C status in fish. However, the highest tissue concentration of AA was recorded with 500mg AA/Kg, which may reflect the storage capacity of the organs.

2- The second experiment:-

After 2 weeks post-infection, 90% of the infected fish fed the AA free diet were dead (Table, 7). Mortality rates were lower gradually with increasing of ascorbic acid concentration in the diets till reach 10% in fish group fed on 1000 mg AA/Kg diet. Similar result was obtained by Wahli et al. (1986) with rainbow trout infected by *icthyophthirius multifiliis* and supplemented with a megadose of vit.C (5000 mg/kg diet).

The results of pathological examination was cleared that addition of the AA to the diet was minimize the pathological lesions of the infection in the examined organs (liver, spleen, intestine and testis). This results were agreement with Liu et al (1989) and Durve & Lovell (1982), they concluded that addition of AA to the diet of catfish were enhance the disease resistance against the bacterial pathogens. The previous results also clarified that, there were relative increase in the lymphocytic and monocytic cell percentage in the circulation as well as improvement in the lymphocytic population of the spleen in the fish groups fed on the diets supplemented with higher doses of vit.C. This well indicating an enhancement of the immunological status with addition of vit.C to the diets. Similar results were obtained in catfish and rainbow trout fed on diets supplemented with vit.C (Verlhac & Gaboudan, 1990; Li & Lovell, 1985).

The data presented indicate that vit.C has an important influence on tilapia defense mechanisms. Ascorbic acid supplementation enhance resistance of tilapia fish to *pseudomonas* infection.

In conclusion, commercial feed ingredients are almost completely devoid of vit.C, so the vitamin must be supplemented into the practical feeds for normal growth, and to increase the disease resistance and enhance the immune response of the tilapia fish against the bacterial pathogens.

REFERENCES

Agrawal, N.K.and Mahajan, C.L.(1980): Comparative tissue ascorbic acid studies in fishes.J. Fish Biol.,17:135-141.

Al-Amoudi, M.M;El-Nakkadi, M.N. and El-Nouman, B.M.(1992): Evaluation of optimum dietary requirement of vit.C for the growth of O.spilurus fingerlings in water from the Red sea. Aquaculture, 105:165-173.

Andrew, J.W.and Murai, T.(1974): Studies on the vit.C requirements of channel catfish.J. Nutr.,105:557-561.

Association of Official Analytical Chemists (1984): Methods of analysis.Washington, DC.

Bancroft, J.D. & Stevens, A. (1993): Theory and practice of histologic techniques. 3rd edition, Longman Group Limited, pp. 113.

Beisel, W.R.(1982): Single nutrients and immunity.Am.J.Clin.Nutr.,35:423-428.

Blazer,V.S.; Wolke,R.E. (1984): The effects of alpha-tocopherol on the immune response and non-specific resistance factors of rainbow trout.Aquacult., 37:1-8.

Chatterjee, I.B.(1978): Ascorbic acid metabolism. World Rev. Nutr. Diet. 30:69-87.

Coles, E.H. (1986): Veterinary clinical pathology. 4th edition, W.B.Saunder Company Philadeliphia & London.

Dabrowski, K.; Hinterleitner, S.; Sturmbauer,C.; El- Fiky, N. and Wieser, W. (1988): Requiremens of carp larva for vit.C.Aquacult.,72:295-306.

Dabrowski, K. (1990): Ascorbic acid status in the early life of whitefish (Coregonus lavaretus L.). Aquacult., 84:61-70.

Duncan, D.(1955): Multiple range and multiple F-tests.Biometrics, 11:1-42.

Durve,V.S.and Lovell, R.T.(1982): Vit.C and disease resistance in channel catfish. Can. J. Fish. Aquat.Sci.,39:948-953.

Fenster, R.(1987): Vit.C in fish nutrition. Roche Seminar on vitamin nutrition in fish. Trondheim, Aug., 1987.

Gatlin, D.M.and Wilson, R.P.(1986): Characterization of iron deficiency and dietary requirement for channel catfish.Aquacult., 52:191-198.

Grant,B.F.; Seib,P.A.; Liao,M.L. and Corpron,K.E.(1989): Polyphosphorylated L-ascorbic acid: a stable form of vit.C for aquaculture feeds. Aquacult.,15:75-81.

Halver, J.E. (1979): Vitamin requirements in fin fish. In J.E.Halver and K.Tiews (Editors), Proc.World Symp.on Fin Fish Nutrition and Fish Feed Techn., Hamburg, 1978.

Halver, J.E.(1989): The vitamins. In: J.E.Halver (Editors), Fish Nutrition Academic Press,New York,pp 32-109.

Halver, J.E.; Ashley, L.M. and Smith, R.R. (1969): Ascorbic acid requirements of coho salmon and rainbow trout. Trans.Am.Fish Soc., 98:762-771.

Hilton, J.W.; Cho, C.Y. and Slinger, S.J.(1977): Evaluation of the ascorbic acid status of rainbow trout. J.Fish.Res.Board Can., 34:2207-2210.

Hilton, J.W.; Cho, C.Y.and Slinger, S.J.(1978): Effects of graded levels of supplemental ascorbic acid in practical diets for rainbow trout. J.Fish.Res. Can., 35:431-436.

Hornig, D.; Glatthaar, B.and Moser,U.(1984): General aspects of ascorbic acid function and metabolism. In Wegger, I.; Tagwerker, F.J.; Moustgaard, J.(Editors): Ascorbic acid in domestic animals proceedings. The Royal Danish Agric. Soc., Copenhagen.pp 70-75.

Ikeda, S.and Sato, M.(1964): Biochemical studies on L-ascorbic acid in aquatic animals. III.Synthesis of ascorbic acid in carp. Bull.Jpn.Soc.Fish.,30:365-369.

Jauncey, K and Ross, B.(1982): A guide to Tilapia feed and feeding. Inst. of Aquaculture, Univ.of Stiriling, Stirling, 111pp.

Jauncey, K.; Soliman, A. and Roberts, R.J. (1985): Ascorbic acid requirements in relation to wound healing in the cultural tilapia (O.niloticus). Aquacult.Fish Manag., 16(12): 139-149.

John, T.M.; George, J.C.; Hilton, J.W.and Slinger, S.J.(1979): Influence of dietary ascorbic acid on plasma lipid levels in the rainbow trout. Int.J.Vit.Nutr.Res., 49:400-405.

Kitamura,S.;Ohara,S.;Suwa,T.and Nakagawa,K.(1965): Studies on vitamin requirements of rainbow trout.I.On the ascorbic acid. Bull. Jpn. Soc. Sci. Fish., 31:818-82

Li, Y. and Lovell, R.T. (1984): Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J. Nutr. 115:123-131.

Li,Y.and Lovell,R.T.(1985): Elevated levels of dietary ascorbic acid increase immune responses in channel catfish. J.Nutr.,115:123-131.

Lim,C. and Lovell, R.T.(1978): Pathology of the vitamin C deficiency syndrome in channel catfish. J.Nutr.,108:1137-1146.

Liu, P.R.; Plumb, J.A.; Guerin, M. and Lovell, R.T. (1989): Effect of megalevels of dietary vitamin on the immune response of channel catfish (Ictalurus punctatus) in ponds. Disease Aquatic Organ.,7:191-194.

Lovell, R.T.(1973): Essentiality of vit.C in feeds for intensively fed caged catfish. J.Nutr.,103:134-138.

Lovell, R.T.(1984): Use of soybean products in diets for aquaculture species .Res. Highlights, Amer. Soybean Assoc., February, 15-24.

Lovell, R.T. (1989): Nutrition and feeding of fish. Academic Press, New York, pp 54-60.

Mahajan, C.L. and Agrawal, N.K.(1980): Nutritional requirement of ascorbic acid by Indian major carp during early growth. Aquacult.,19:37-48.

Miyazaki, T.; Plumb, J.A.; Li, Y.P.and Lovell, R.T.(1985): Histopathology of broken back syndrome in channel catfish.J.Fish Biol.,26:647-655.

Murai, T.; Andrews, I.W. and Bauernfiend, J.C.(1978): Use of L-ascorbic acid, ethocel- coated ascorbic acid and ascorbate 2-sulphate in diets for channel catfish. J.Nutr., 108:1761-1766.

National Research Council (1993): Nutrient requirements of fish and shellfishes. Washington , DC. National Academy Press.

Navarre, O. and Halver, J.E.(1989): Disease resistance and humoral antibody production in rainbow trout fed high levels of vit.C. Aquacult., 79:207-221.

Paterson, W.D.; Lall, S.P.; Audrie, D.; Greer, P.; Greenham, G.; Poy, M.(1985): Prevention of disease in salmonids by vaccination and dietary modification. Fish Pathol., 20:427-432.

Poston, H. (1967): Effect of dietary L-ascorbic acid on immature brook trout. N.Y.State Conserv.Dep.Fish.Res.Bull., 30:46-51.

Sato, M.; Kondo, T.and Yoshinaka, R.(1983): Effect of water temperature on the skeletal deformity in ascorbic acid deficient rainbow trout. Bull. Jap. Soc. Sci. Fisheries, 49: 443-446.

Sato, N.; Yoshinaka, R. and Ikeda, S. (1978): Biochemical studies on L-ascorbic acid in aquatic animals. XI.Dietary ascorbic acid requrirement of rainbow trout for growth and collagen formation. Bull.Jpn.Soc.Fish., 44:1029-1035.

Schuep, W.; Vuilleumier, J.P.; Gysel, D.and Hess, D.(1984): Determination of ascorbic acid in fluids,tissues and feedstuffs.In Wegger, I.;Tagwerker, F.J.; Moustgaard,J. (Editors): Ascorbic acid in domestic animals proceedings. The Royal Danish Agric.Soc., Copenhagen.pp 50-55.

Skelbaek, T.; Anderson, N.G.; Winning, M.and Westergaard, S.(1990): Stability in fish and feed bioavailability to rainbow trout of two ascorbic acid forms. Aquacult., 84: 335 -343.

Soliman, A.K.; Jauncey, K.and Roberts, R.J.(1985): Qualitative and quantitative identification of L-gulonolactone oxidase activity in some teleosts. Aquacult. Fish Manag.16:249-253.

Soliman, A.K.; Jauncey, K.and Roberts, R.J.(1986): The effect of varying forms of dietary ascorbic acid on the nutrition of juveniles tilapia (O.niloticus). Aquacult., 52:1-10.

Stickney,R.R.;McGeachin,R.B.;Lewis,D.H.Marks,J.;Riggs,A.;Sis,R.F.;Robinson,E.H.and Wurt,W.(1984): Responses of T.aurea to dietary vit.C.J.World Maricult. Soc.,15: 179-185.

Tolbert, B.M.(1979): Ascorbic acid metabolism and physiological function.Int. J.Vit.Nutr. res. Suppl., 19:127-142.

Verlhac, V. and Gabaudan, J. (1990): Effect of a high dietary dose of ascorbate-2- monophosphate on the immune response of rainbow trout. In: Ascorbic Acid in Domestic Animals Symp., Switzerland, Oct.,9-12, pp. 456-461.

Wahli, T.W.; Meier, T. and Pfister, K. (1986): Ascorbic acid induced immune-mediated, decrease in mortality in Ichthyophthirius multifilis infected rainbow trout. Acta Tropica, 43:287-289.

Weber, G.M.(1997): Vitamins and the immune response of farm animals. Hofmman La-Roche Report.

Wilson, R.P.and Poe, W.E.(1973): Impaired collagen formation in the scorbutic channel catfish. J.Nutr.,103:1359-1364.

LIST OF FIGURES

Fig.1:- Liver showing diffuse fatty change of the hepatocyte (H&E, 10x25).

Fig.2:- Liver showing fatty degeneration of the hepatocyte (H&E, 10x25).

Fig.3:-Liver showing granular degeneration and swelling of the hepatocytes (H&E,10x25).

Fig.4:- Liver from the fish in the 6th group, showing more or less normal histological appearance (H&E,10x25).

Fig.5:- Spleen showing severe exhaustion of the lymphocyte. The splenic tissue appear as a mesh of reticular fibre denuded from lymphocyte (H&E, 10x10).

Fig.6:- Spleen showing moderate exhaustion of the lymphocyte (H&E, 10x10).

Fig.7:- Spleen from the fish in the 5th group showing increase population of the lymphocyte (H&E, 10x10).

Fig.8:- Spleen from the fish in the 6th group showing normal histological appearance (H&E, 10x10).

Fig.9:- Intestine showing necrosis and desqumation of the epithelium in the tips of the villi (H&E, 10x10).

Fig.10:- Intestine from the fish in the 6th group showing normal histological patterns (H&E, 10x25).

Fig.11:- Testis showing degeneration and necrosis of spermatogenic cells in the semineferous tubules (H&E, 10x25).

Fig.12:-Testis showing focal degeneration in the spermatogenic cell (F) in the semineferous tubules (H&E, 10x25).

Table 1: Composition of the basal diet used in the study.

Ingredients	%
Physical comp.:	
Fish meal	24
Soybean oil meal	32
Corn, ground	35
Wheat bran	3
Fish oil	3
Vitamin mixture*	1
Mineral mixture**	2
Chemical comp.:	
Protein (%)	32.10
Digestible energy (Kcal/Kg diet)	3147

*Vit.mix.(without vit.C): each 100g contain: 1000,000 IU vit.A; 0.25g thiamine chloride; 0.25g vit.B2; 0.20g vit.B6; 0.50mg cyanocobalamine; 0.50g Ca pantothenate; 75mg folic acid; 0.20g menadione; 0.25g para-aminobenzoic acid; 10g inositol; 1g niacin; 30mg biotine; 20g choline chloride.

**Min.mix.(mg/Kg): 62 manganese; 24.8 zinc; 12.4 iron; 6.2 copper; 1.9 iodine; 0.3 cobalt; 155.1 calcium.

Table 2: Deformities and mortality percentages of tilapia fingerlings fed on different dietary ascorbic acid levels (in first experiment).

Dietary AA (mg/Kg)	Physical deformity %	spinal deformity %	mortality %
0	50	20	45
50	7	0	5
150	5	0	3
250	9	0	7
500	6	0	5
1000	3	0	3

Table (3): Performance of tilapia fingerlings fed on different dietary ascorbic acid levels (in first experiment).

Dietary AA (mg/Kg)	Initial weight (g)	Final weight (g)	Weight gain (g)	Feed intake (g)	F.C*	C.F*	SGR*
0	20.13±1.03	34.28±1.10^{c}	14.15±1.10^{c}	42.45±1.40^{c}	3.00	2.17	0.23
50	20.40±1.05	56.71±1.15^{b}	36.31±1.15^{b}	64.99±1.50^{b}	1.79	1.87	0.42
150	20.34±1.05	64.62±1.35^{a}	44.28±1.25^{a}	75.28±1.60^{a}	1.70	1.82	0.48
250	20.12±1.01	59.39±1.20^{a}	39.27±1.20^{a}	69.12±1.50^{a}	1.76	1.81	0.45
500	20.10±1.01	60.30±1.25^{a}	40.20±1.18^{a}	69.95±1.40^{a}	1.74	1.84	0.46
1000	20.60±1.07	63.92±1.30^{a}	43.32±1.20^{a}	74.08±1.50^{a}	1.71	1.85	0.48

Figures in the same column having the same superscripts are not significantly different (P<0.05).

*F.C= feed conversion; C.F= condition factor; SGR= specific growth rate.

Table (4): Whole body analysis of tilapia fed on different dietary ascorbic acid levels (on DM basis) in first experiment

Dietary AA (mg/Kg)	dry matter (%)	crude protein (%)	crude fat (%)	crude ash (%)
Blank group	25.80	73.25	18.53	15.78
0	27.82±1.05^{c}	59.31±1.20^{c}	20.16±1.02^{b}	13.19±1.01^{d}
50	29.40±1.05^{b}	63.20±1.40^{b}	21.14±1.04^{b}	16.46±1.05^{b}
150	28.15±1.07^{b}	62.84±1.35^{b}	20.78±1.02^{b}	15.70±1.03^{c}
250	30.35±1.20^{b}	64.32±1.40^{b}	23.10±1.03^{a}	17.10±1.05^{b}
500	30.18±1.15^{b}	64.91±1.45^{b}	22.42±1.02^{a}	17.33±1.03^{b}
1000	32.20±1.25^{a}	67.11±1.48^{a}	24.51±1.05^{a}	18.82±1.07^{a}

Figures in the same column having the same superscripts are not significantly different (P<0.05).

Table (5):Dry matter and protein parameters of tilapia fed on different dietary ascorbic acid levels in first experiment.

dietary AA (mg/Kg)	DM parameters		Protein parameters				
	DM retention (g)	DM digest. (%)	Protein intake (g)	protein retention (g)	PER	PPV	protein digest. (%)
0	4.38	54.10	12.64	1.88	1.12	14.87	60.12
50	11.51	68.40	19.35	6.76	1.88	34.94	82.84
150	13.03	70.35	22.42	7.65	1.98	34.12	83.15
250	12.86	70.52	20.58	7.81	1.91	37.95	83.50
500	13.04	70.84	20.83	8.03	1.93	38.55	83.78
1000	15.42	72.13	22.06	10.03	1.96	45.47	84.65

Table (6): Tissue concentration of AA (mg/100g wet tissue) in tilapia fed on different dietary ascorbic acid in first experiment.

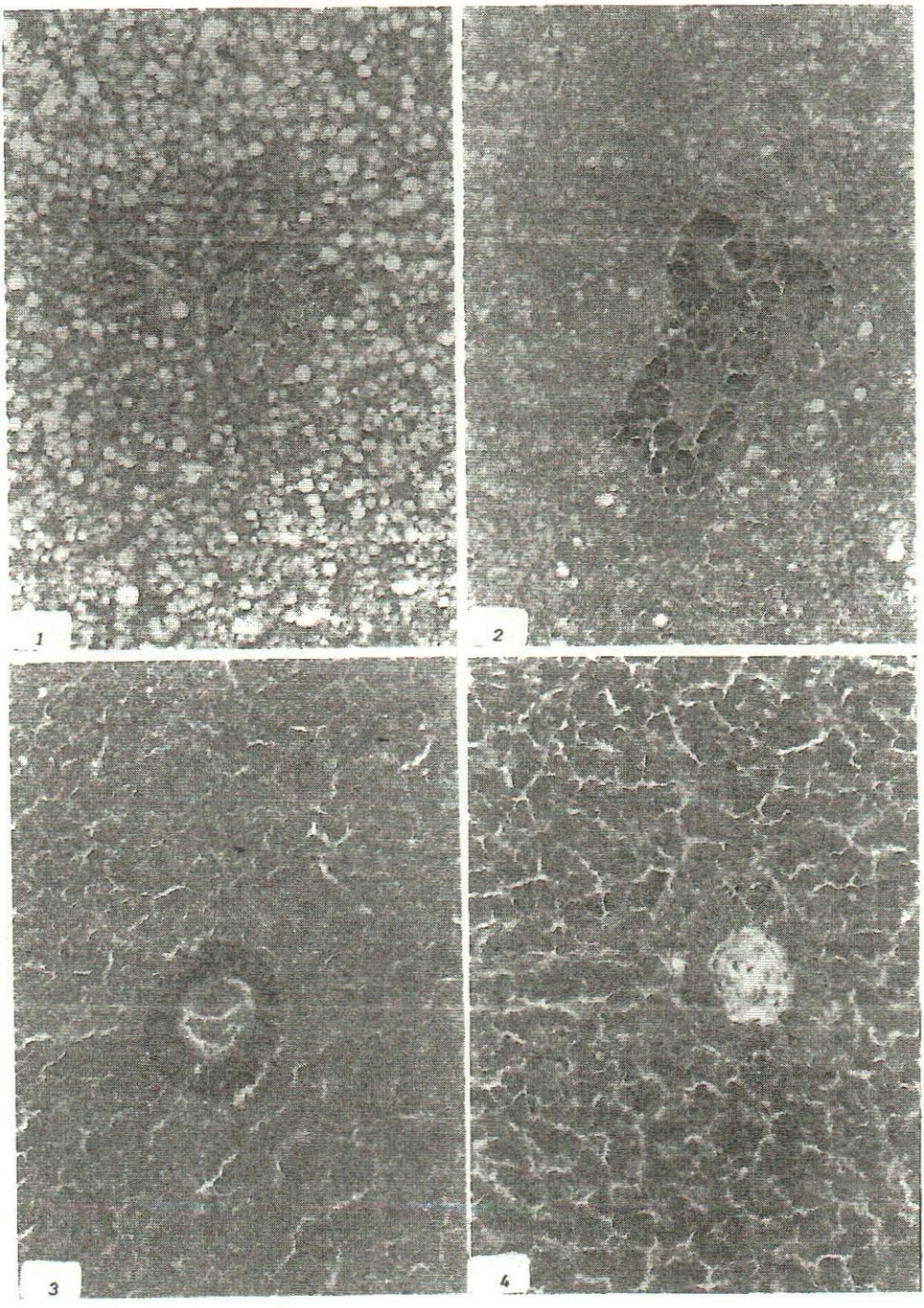
dietary AA (mg/Kg)	Spleen mg/100g	Liver mg/100g	Muscle mg/100g	Kidney mg/100g
0	4.8	7.5	1.1	3.1
50	12.5	22.3	3.3	10.9
150	67.23	52.0	15.2	11.7
250	75.31	54.1	25.1	11.0
500	81.40	70.3	27.4	15.6
1000	45.20	50.0	24.2	22.3

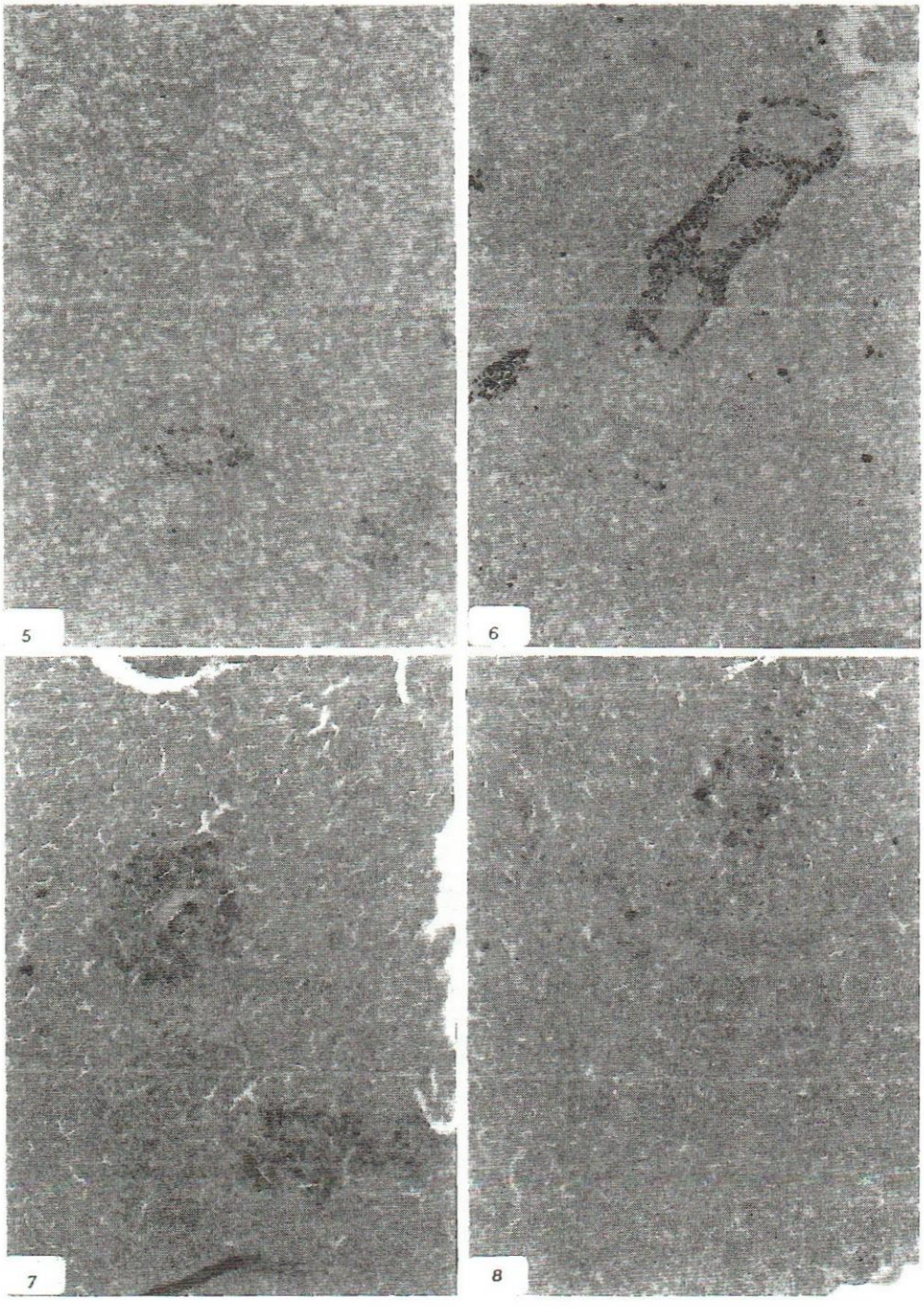
Table (7):-Mortality percentages of tilapia fed on different dietary AA after 2 weeks post-infection with *P.fluorescens* in second experiment.

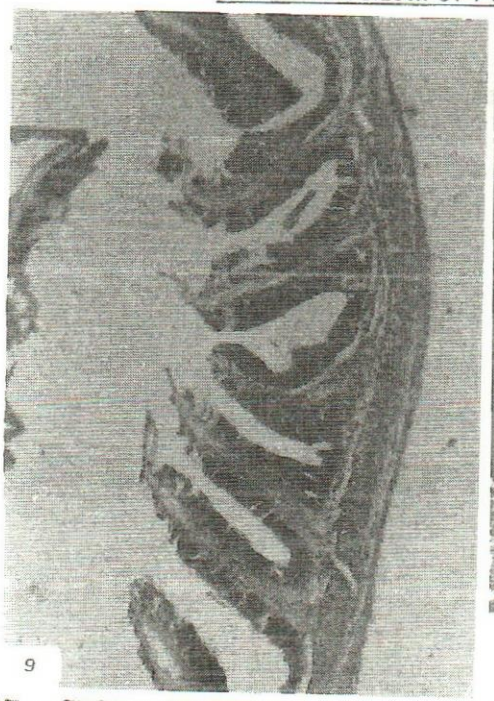
Groups	Dietary AA mg/kg diet	No. of fish	No. of dead fish	Mortality %
1st group	**0**	20	18	90
2nd group	**50**	20	13	65
3rd group	**150**	20	9	45
4th group	**250**	20	5	25
5th group	**500**	20	3	15
6th group	**1000**	20	2	10

Table (8):-Effect of ascorbic acid on hematology of tilapia subjected to *Pseudomonas fluorescens* infection.

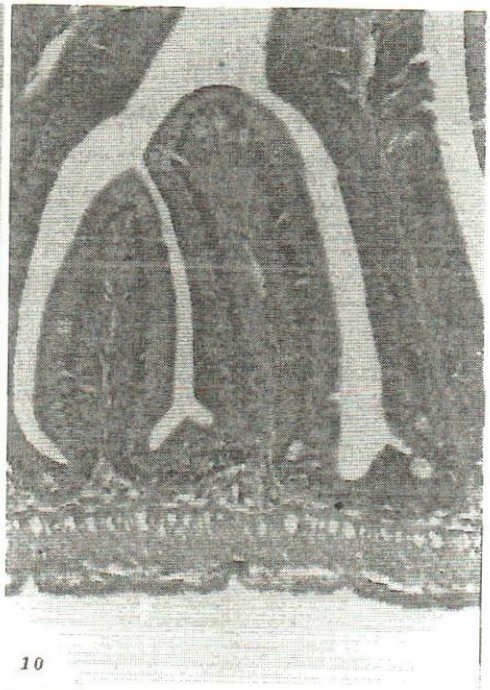
Groups	Dietary AA mg/kg diet	RBCs $\times 10^6$	WBCs $\times 10^3$	Lymphocyte %	Heterophil %	Monocyte %	Eosinophil %	Basophil %
1st group	**0**	4.12±0.13	1.50±0.81	48.66±3.38	43.33±5.17	7.66±2.03	1.34±0.012	---
2nd group	**50**	4.22±0.01	3.50±0.70	56.33±1.20	34.66±1.20	7.66±0.33	1.00±0.000	---
3rd group	**150**	5.36±0.20	3.70±0.13	61.27±0.04	28.23±0.11	10.10±0.13	1.40±0.009	---
4th group	**250**	6.41±0.03	5.42±0.11	67.33±2.03	22.00±1.73	10.33±0.33	1.00±0.000	---
5th group	**500**	6.21±0.06	5.53±0.06	71.50±0.13	16.00±1.05	12.28±0.41	1.22±0.001	---
6th group	**1000**	6.12±0.12	5.51±0.02	69.66±1.33	20.66±2.18	8.66±0.88	1.00±0.000	---


1
2
3
4

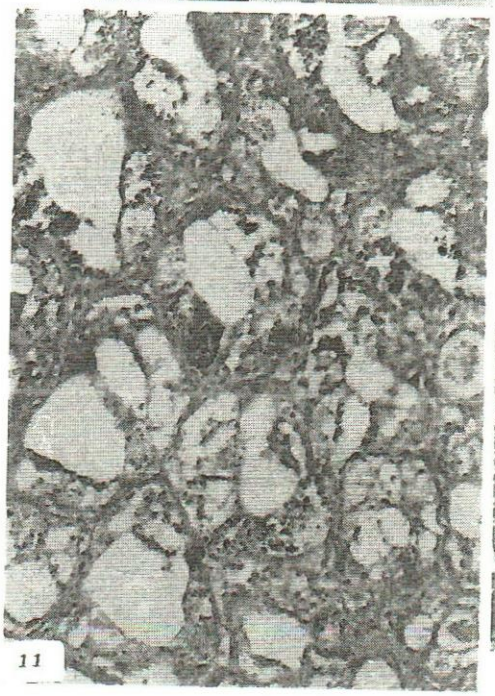

5
6
7
8



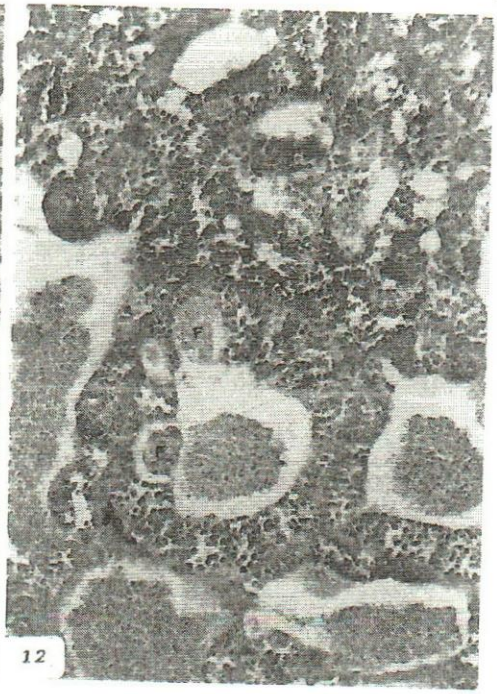
9



10



11



12