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SYNERGISTIC REACTIONS BETWEEN VITAMIN E AND SELENIUM IN DIETS OF HYBRID TILAPIA *(OREOCHROMIS NILOTICUS* X *OREOCHROMIS AUREUS)* AND THEIR EFFECT ON THE GROWTH AND LIVER HISTOLOGICAL STRUCTURE

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ABSTRACT

Eight experimental diets representing two levels of vitamin E (low 100 IU /kg diet and high 300 IU/kg diet) with four levels of selenium (0;2 ; 4 ; 8 mg/kg diet) were tested on hybrid tilapia (*O. niloticus X O. aureus*) for a period of 120 days . The study was performed in outdoor cement ponds, each divided to units by nets with an area of $10m^3$ each. Each treatment was performed in duplicates, using 40 experimental fish with an initial weight of 48.83 g on the average . After the adaptation period, the fish were fed on the experimental diets at a rate of 3% of the treatment biomass dialy, divided into two equal portions at 10. 0 a.m and 2 p.m. Water temperature ranged between 28.7 to 31.5 ^{0}C during the experimental period. Water quality parameters (dissolved oxygen, ammonia pH) were within the permissible levels for tilapia. Results obtained are summerized in the following :

1-Hybrid tilapia fed on 2mg Se with 300 IU vitamin E/kg diet had significantly higher ($p < 0.05$) specific growth rate (SGR), feed conversion efficiency and also improved feed conversion ratio than that fed on the other diets.

2-At constant dietary vitamin E level, total serum protein increased with increasing Se level up to 8 mg/ kg diet.

3-Fish fed on high level of supplemented vitamin E (300 IU/kg) diet without selenium deprivation showed increased growth rates and

improved feed conversion rate than that fed on the low vitamin E (100IU/kg dry diet).

4 - The histological investigation showed remerkable effects in the liver tissue of hybrid tilapia fed on dietary Se- supplemented level up to 8mg/kg diet with low vitamin E (100IU/kg dry diet) with different degrees of injuries, including blood vessels, hemorrhages and degeneration in hepatic cells, dilatation, odema and hemosidren, hemolysis, necrosis and activation in nuclear division.

This study suggests that supplementation of Vit. E 300IU/kg with selenium up to 2 mg / kg dry diet for hybrid tilapia is warranted to increase growth rate and prevent fish from increased concentration of waterborne Se up to toxicity levels.

INTRODUCTION

Human population explosion poses many problems of water contamination, often resulting in the direct or indirect damage and possible destruction of aquatic populations. Interest in the effects of acute and chronic selenium toxicity in aquatic organisms has recently increased, probably because of the localized rise in the waterborne selenium levels such as in the Great lakes which may be related to industrial pollution (International Joint Commission, 1976). Halverson *et al.* (1966) found that symptoms of chronic selenium toxicity included impaired liver function, decreased liver weight , and depressed hemoglobin levels. Acute toxicity of waterborne selenium occurs at concenterations of 4 - 20 mg/ liter. whereas symptoms of chronic toxicity occur at concentrations between 60 and 130 µg/ liter (Huckabee and Griffith, 1974). Hilton *et al.* (1980) and Hodson. *et al.* (1980) reported that the chronically toxic waterborne concenteration is 100-1000 times the background concenteration of surface water, but the toxic dietary concenteration is only 10 times the normal dietary level. Chronic dietary selenium toxicity occurs in rainbow trout (*Salmo gairdneri*) at 13 µg selenium per gram dry feed (Hilton *et al.*, 1980). Hodson and Hilton (1982) found that prolonged ingestion of as little as 3µg selenium per gram dry feed may ultimately be toxic. Hilton and Hodson, 1983 and Gatlin and Wilson (1984) suggested that levels above 13-15 mg selenium / kg dry feed for trout, can be toxic, though trout receiving over 10mg Se/kg developed renal calcinosis.

On the other hand, selenium has long been recognized as an essential dietary nutrient in fish (Watanabe *et al.*, 1997). It has been found to be an integral component of glutathione peroxidase. This enzyme,

protects cells and membranes from oxidative damage by destroying hydrogen peroxide and hydroperoxides employing reducing equivalents from glutathione (Bell and Cowey, 1985). The same authors, indicated that selenium supplied as selenite (inorganic selenium compounds) was a better source for plasma glutathione peroxidase. Selenium compounds are also capable of protecting from the toxicity of heavy metals, such as cadmium and mercury (Watanabe *et al.*, 1997). Vitamin E on the other hand is a membrane associated antioxidant and/ or scavenger of free radicals. Accordingly, selenium has been used in conjunction with vitamin E for avoidance of nutritional muscular dystrophy. The two nutrients complement each other in their activity and protect biological membranes against lipid oxidation (Combs and Combs, 1986).

The differences noted in the interaction between vitamin E and selenium in fish, may also be affected with species and environmental conditions (Cowey *et al.*, 1984; Bell *et al.*, 1985). Poston *et al.* (1976) observed that both vitamin E (0.5 IU/ g diet) and selenium (0.1 µg /g diet) were required to prevent nutritional muscular dystrophy in Atlantic salmon. Hilton *et al.* (1980) suggested that a dietary selenium level of 0.07µg /g dry feed with a waterborne selenium level of 0.4 µg/ liter and a dietary vitamin E level of 0.4 IU/ g dry diet was sufficient to prevent frank selenium deficiency symptoms in rainbow trout. Gatlin and Wilson (1984) found that the selenium requirement of fingerling channel catfish *(Ictalurus punctatus)* was between 0.1 and 0.5 mg Se/kg dry diet. Salte *et al.* (1988) estimated a dose of 50 mg/kg body weight vitamin E and selenium at 0.12 mg/kg in diet Atlantic salmon to prove whether surplus vitamin E and selenium could prevent "Hitro disease" or hemorrhagic syndrome. NRC (1993) mentioned that the requirement of dietary vitamin E for most fish ranges from 30 to 100 IU/ kg dry diet. In this connection, Shiau and Shiau (2001) reported that a dietary vitamin E of 60-67 IU/kg dry diet has been required for hybrid tilapia. However, Huang *et al.* (2004) found that a dietary vitamin E content of ≥200 IU/ kg or higher would enhance the ability of hybrid tilapia muscle against lipid peroxidation during storage.

Liver tissue was used as the primary reference material for selenium analysis, since this organ preferentially accumulates selenium in exposed fishes (Hodson *et al* 1980.,).

The present study was carried out to investigate the effect of vitamin E in reducing the toxicity of waterborne selenium in diets of hybrid tilapia *(Oreochromis niloticus X Oreochromis aureus)* .

MATERIALS AND METHODS

1- Experimental System:

The experiment was carried out in outdoor cement ponds subdivided by netting to 16 rearing units (10 m^2/ unit with 1m depth each). The pond series were located at the campus of the National Institute of Oceanography and Fisheries. The experiment lasted for 120 days during the period from 7 June to 4 October,2004.

2- Experimental design and diets:

To explore more fully the interrelationships between selenium and vitamin E on male hybrid tilapia *(Oreochromis niloticus* X *Oreochromis aureus)* a 2x4 factorial experiment with two vitamin E levels (α-tocopheryl acetate) was added to the diets to obtain the final content 100 or 300 IU/kg dry diet, within each vit. E level, four selenium levels (Sodium selenite, Na_2 SeO_3) 0, 2, 4 and 8 mg/kg dry diet were tested (Table 1).

The selenium content of the basal diet was determined by the method of Hoffman *et al.* (1968). Vitamin E level of the basal diet was analyzed by High Performance Liquid Chromatography (Miller *et al.*, 1994).

The basal diet was formulated from conventional ingredients to contain 30% crude protein and 3240 kcal metabolizable energy/ kg dry diet, according to Chou and Shia (1996), for male hybrid tilapia.

The tested diets were analyzed for crude protein, ash and moisture according to methods described by AOAC (1995), for lipid by the method of Bligh and Dyer (1959). and Both supplemented selenium and vitamin E were added separately to the basal diet (Table 2) and compensated by reductions in the amount of wheat bran.

The water quality parameters were carried out in rearing units as described by APHA (1992). Approximately, 1/2 of the water in each cement pond was replaced with aerated freshwater (from River Nile) weekly with complete change of water every two weeks to maintain water quality at the permissible levels (dissolved oxygen was > 5.8 mg/l; ammonia < 0.25 ppm) throughout the experimental period. Water temperature ranged from 28.7 to 31.5C°; and pH from 7.4 to 8.3 respectively.

3- Fish and feeding trials:

Prior to the start of experiment, hybrid tilapia (male fish) were purchased from the local tilapia farm and acclimated in outdoor cement (rearing unit) for two weeks and fed on a basal diet (Table 2) without

vitamin E and selenium supplementation . The fish were stocked at a density of (40) per enclosure with an average initial body weight of 48.83 ± 0.7/g. ($\bar{X}$ ± S.E.).

Each experimental diet was tested in duplicate rearing units for 120 days. Hybrid tilapia were fed their corresponding experimental diets at a rate of 3.0% of wet body weight per day in two equal partitions at 10.0 a.m and 2.p.m. Fish in each rearing unit were counted and the experimental groups were weighed every 15 days. No feeding was done on sampling days and the daily ration was adjusted according to the new group weight .

Analytical methods:

At the termination of the feeding trial, the hybrid tilapia were counted and weighed individually. Blood samples were withdrawn from the caudal vein of ten fish from each treatment. Aliquots of blood samples were used to determine blood characteristics; haematocrit value (Brown , 1980) and haemoglobin concentration by the cyanmethemoglobin (Larson and Snieszlco, 1961). The remaining samples were centrifuged at 3000 rpm for 20 minutes. Serum samples were collected and stored for further analysis at 4°C to determine total serum protein by the method of Henery (1964). From each treatment ten fishes, whole muscle fillets were analyzed for ash, crude protein and moisture by AOAC (1995) . Crude lipid content by the method of Bligh and Dyer (1959) and muscle gross energy as described by NRC (1983).

Histological method

Moreover, tissue specimens from the liver of the studied fish were fixed in 10% phosphate buffer formalin, followed by dehydration through an ascending series of ethyl alcohol. Finally specimens were embedded in paraffin and cut to sections of 5 microns, using Euromex Holland Microtome and stained according to hematoxylin and eosin method (Pearse, 1972). The sections were examined histologically by a light microscope and photographed by a microscopic camera.

Statistical methods:

Data were subjected to a factorial analysis of variance (Steel and Torrie, 1980). Treatment means were tested by using Duncan's multiple-range test ($p<0.05$), (Duncan, 1955).

RESULTS AND DISCUSSION

a) Growth performance :-

Growth performance and feed efficiency results of the hybrid tilapia *(Oreochromis niloticus* X *Oreochromis aureus)* fed on different levels of vitamin E (high, 300 and low 100 IU/kg dry diet) and selenium (0, 2, 4 and 8 mg/kg dry diet) are presented in Table (3). Weight gain (WG), specific growth rate (SGR) and feed efficiency of the fish fed on diets containing high levels of vitamin E (300 IU/kg dry diet) and supplemented with selenium (2 mg/kg dry diet) were significantly higher ($p<0.05$) than those fed on the other diets (Tables 3 & 4). However, hybrid tilapia given diet depleted of Se (0 supplement), had significantly lower mean final body weight, specific growth rate and high feed conversion ratio (Tables, 3& 4). It is well established that selenium and vitamin E have different but complementary biochemical functions which may allow these nutrients to interact physiologically (Gatlin *et al.*, 1986). The same authors recorded that catfish fed the basal diet without supplemental selenium and vitamin E had significantly lower weight gain and feed efficiency than those fed other diets (supplemented with 0.2 mg/kg selenium, 50 mg/kg vitamin E or both). Though, Gatlin *et al.* (1984) showed that catfish fed 0 and 0.1 mg/kg supplemental selenium had significantly suppressed growth rates but no gross pathological changes were evident.

Lower growth performance of hybrid tilapia fed 0 supplemented selenium in treatment 1 and 5 (Table 3) may(Huang and Huang, 2004; NRC 1983) be attributed to decreased selenium in basal diet than the requirement.

Moreover, results presented in Fig. (1) indicate that growth performance decreased as the level of selenium in the diet decreased from 4 to 0 mg/ kg dry diet regardless of vitamin E levels. Similar trends have been reported by Bell *et al.* (1987), who used two groups of Atlantic salmon *(Salmo salar)* of mean weight 6.0g and were given diets of differing selenium content (deficient 0.017 mg Se/kg diet; supplemented 0.944 mg/kg diet) and adequate vitamin E content (40 mg α - tocopheryl acetate / kg) for 28 weeks. The final weight of salmon given the Se-supplemented diet were significantly greater than those of the Se-deprived diet. Contrary to that, with rainbow trout, Bell *et al.* (1985 & 1986) observed a significant reduction in final weight achieved by Se-deficient fish. This may be due to the lower level of dietary vitamin E supplied (which might render the fish less able to retain liver Se) which reflect the

synergism already noticed among both Vit. E and Se. It may, on the other hand, reflect a higher requirement for Se by Atlantic salmon (Bell *et al.*, 1987). This latter view is supported by the fact that glutathione peroxidase activity in control salmon is almost 10 times that in rainbow trout. Munro *et al.* (1984) and Ferguson *et al.* (1986) suggested that a rapid loss of weight in Atlantic salmon *(Salmon salar)* may be due to the depletion of antioxidant factors, notably Se and vitamin E, that has been implicated in the development of the pancreas disease condition.

Hybrid tilapia reared on the highest dietary level of selenium (8 mg/kg dry diet) had a lower growth performance and feed efficiency than the remaining groups (2 and 4 mg Se/kg dry diet) regardless of vitamin E level (Fig. 1). Similar findings were early reported by Hilton *et al.* (1980) for rainbow trout *(Salmo gairdneri)*, that no significant differences ($p > 0.05$) were detected in the body weight, feed gain ratio and number of mortalities in groups fed unsupplemented selenium in comparison with groups fed supplemented selenium (0.1 to 4.29 μg/g diet). Therefore, they showed that a dietary selenium of 15 μg/g dry diet would appear to induce significantly lower body weight, a higher feed gain ratio and a higher number of mortalities than the remaining supplemented selenium levels (0.1 to 4.29 μg/g diet). The same authors suggested that chronic dietary selenium toxicity was found to occur in trout fed diets containing approximately 13 μg selenium/ g dry feed. Similar findings were reported by Hilton and Hodson (1983) for rainbow trout *(Salmo gairdneri)* . They revealed that trout reared on the high dietary levels of selenium (10 μg selenium per gram of diet for 16 weeks), showed significantly reduced body weight and impaired efficiency. It is possible that excess selenium in diet may interfere with copper transport or excretion perhaps through substitution of selenium for sulfur in metallothionen (Hilton and Hodson, 1983). In this connection, Mc Connell *et al.* (1974) showed that selenium has been used to substitute for sulfur in protein synthesis, and the resultant selenoprotein may not have the same biological activity as the equivalent sulfur protein. Moreover, Hilton and Hodson (1983) found that trout reared on high dietary selenium diets (10 μg/g diet) had an increased incidence of renal calcinosis. Smart *et al.* (1979) and Cowey *et al.* (1979) showed that the etiology of renal calcinosis is not completely known and a number of diverse factors such as dietary mineral imbalances and water chemistry may increase the incidence of calcinosis in trout. Though, Hilton *et al.* (1982) showed that with increasing dietary selenium, stable selenium

concentration factor, (CF = ratio of tissue to dietary selenium) decreased. This may have been due to a progressively less efficient uptake or a progressively more efficient elimination. Moreover, Hodson *et al.* (1980), and Hilton *et al.* (1980) mentioned that the high concentration of selenium was in liver, after exposure to both dietary and waterborne selenium. They suggested a role for this organ in storage. Steinberg *et al.* (1968) emphasized on the hepatic conversion of inorganic selenium to organic forms which are more easily excreted. Therefore, the source of selenium (dietary vs waterborne) may influence excretion rates by affecting the form of selenium stored in tissue.

In this connection, Lin and Shiau (2005) showed that weight gain and feed efficiency were highest ($P < 0.05$) in Juvenile grouper, *(Epinephelus malabaricus)* fed diet with 0.11 mg Se/kg diet, followed by fish fed diets with 0.21, 2.02 and 2.70 mg Se/kg diet and lowest in fish fed diet with 4.0 mg Se/kg diet. The different quantified requirement to fish from Se may be to different forms of Se used in fed fish (selenomethionine or sodium selenite) (Lin and Shiau, 2005). Organic Se (selenomethionine) has been reported to have higher bioavailability than the inorganic Se (sodium selenite) for Atlantic salmo (Lorentzen *et al.*, 1994) and channel catfish (Wang and Lovell, 1997).

However, results presented by Kim *et al.* (2003) indicated that an excessive level of dietary Se (from 0.2mg to 0.5mg/kg diet) did not enhance growth performance in Nile tilapia.

As given in Table (3), growth performance for hybrid tilapia fed on supplemented vitamin E high level 300 IU/kg dry diet and deficient selenium levels (treat 5), showed significantly increased growth rates compared to those fed diets supplemented with low vitamin E at 100 IU/kg level (treat. 1). These results could be explained by the fact that increased vitamin E in diet may prevent growth depression in hybrid tilapia fed on dietary selenium deficient diet. In this connection, results obtained by Gatlin *et al.* (1986), established that selenium and vitamin E have different but complementary biochemical functions which may allow these nutrients to interact physiologically. Bell *et al.* (1986) obtained better growth rates in rainbow trout *(Salmo gairdneri)* with supplemented selenium than deficient selenium, but no significant differences in both treatments was recorded, when diets contained an adequate amount of vitamin E. Cowey *et al.*, (1981) found that no gross selenium deficiency syndrome occurs in the presence of adequate amounts of vitamin E.

In contrast, Gatlin *et al.* (1986) showed that catfish fed the vitamin E deficient diet with supplemental selenium did not exhibit suppressed growth or any consistent gross deficiency signs. The reasons for these differing responses of channel catfish to vitamin E are suggested to be due to genetic differences within this species, that may influence its sensitivity to vitamin E deficiency (Lovell *et al.,* 1984). Though, Gatlin and Wilson (1984) found that growth depression was observed in selenium – deficient catfish yet this was not noted in selenium deficient Atlantic salmon (Poston *et al,* 1976) and rainbow trout (Hilton *et al,* 1980). This may be attributed to specific physiological differences between these fish species. Another possibility is that the low level of dietary polyunsaturated fatty acid (10 g linolenic acid/kg) together with synthetic anti-oxidant used by Bell *et al.* (1985), may explain the lack of pathological changes in *Salmo gairdneri,* A further possible explanation may lie on the fact that Poston *et al.* (1976) used very small fishes. Therefore, the contribution of both dietary and waterborne selenium concentrations should be considered by evaluating the selenium requirement of the fish (Hilton *et al.,* 1980). They showed that trout can readily take up waterborne selenium, that may affect the selenium requirement of the fish. Therefore, it is likely that the trout regulates selenium by excretion through either biliary, respirational (losses by the gills) or urinary routes.

Hybrid tilapia raised on the unsupplemented selenium diet (treat. 1) began to show a higher number of mortalities within low vitamin E level, than fish fed high supplemented vitamin E level with deficient Se (Table 3). These results could be explained by the fact that increased vitamin E up to 300 UI/ kg in dietary hybrid tilapia diet which deficient in Se, may influenc mainly the decreased mortality of fish. Similar trends have been reported by Hilton *et al.* (1980) who observed no mortality in rainbow trout fed a basal diet containing 0.07 mg Se/ kg diet and adequate vitamin E. Also, Gatlin *et al.* (1986) found no mortality in fingerling channel cat fish fed a purified diet alone or supplemented with 0.2 mg/ kg selenium and 50 mg/ kg vitamin E. However, catfish fed the basal diet without supplemental selenium and vitamin E had higher mortalities than those supplemented with increased Se or Vitamin E or both. Bell *et al.* (1987) showed that increased mortality occurred in Atlantic salmon *(Salmo salar)* fed diets deficient in Se (0.017 mg/ kg diet), while no mortality was recorded in fish fed control diet (supplemented with 0.944 mg/ kg Se). Elevated mortality rate was also seen in hybrid tilapia fed

high selenium level 8 mg/ kg diet in both supplemented levels of vitamin E (Table 3). These results agree with findings of Gatlin and Wilson (1984), who showed that increased mortality was observed in swim-up Atlantic salmon fed a selenium level of 15 mg/ kg diet (concluded to be toxic) in spite that vitamin E was adequate in diet.

b) Body composition:

The data on final chemical body composition (muscle) of hybrid tilapia fed varying selenium supplements in this study (Table 5) showed that muscle lipid and ash contents were slightly insignificantly increased ($p > 0.05$) with increasing suppiemented selenium from deficient (0.19 mg Se) to 8 mg/ kg diet and the same trend was obtained with energy retained (Fig. 2). In contrast, muscle protein content was decreased with increased supplemental selenium level up to 4mg Se/kg (Fig. 2). In this connection, Hilton and Hodson (1983) found that the final carcass for rainbow trout fed diet supplemented with from 0 to 10 μg selenium per gram of diet, indicated no significant effect ($p > 0.05$) on crude protein, crude lipid, ash and moisture content of trout reared on different levels of supplemented selenium diets.

c) Hepatosomatic index:

Hepatosomatic index (HIS) was significantly ($P< 0.05$) higher in hybrid tilapia fed the 4 mg Se supplement/ kg diets than the remaining groups (Table 6). However, Hilton and Hodson (1983) found that liver to body weight ratios were not significantly changed ($P>0.05$) in rainbow trout *(Salmo gairdneri)* fed different selenium supplements(0.6, 6.6 and 11.4 μg/ g diets) or with trout fed on selenium supplement from 0 to 15μg/ g diet Hilton *et al.* (1980).

d) Blood parameters:

Hematocrit value was significantly reduced in hybrid tilapia with decreased supplemented selenium level up to 0 (Table 6). Similar trends have been reported by Bell *et al.* (1987) with Atlantic salmon *(Salmo salar);* Bell *et al.* (1986) with rainbow trout *(Salmo gairdneri)* and Gatlin *et al.* (1986) with catfish *(Ictalurus punctatus).* These reports showed that packed cell volume was significantly ($P< 0.05$) lower in the Se deficient fish than in supplemented Se.

These results are in agreement with the findings of Bell *et al.* (1985), who showed that hematocrit values were not significantly affected by giving diets deficient in either vitamin E or Se singly but were reduced to extremely low levels when both nutrients were absent. Gatlin *et al.* (1986) added that combined deficiencies of selenium and vitamin E

caused suppressed hematocrit values in catfish than those fed the diets supplemented with Se 0.2 mg/ kg or 50 mg/ kg diet vitamin E or both.

Results presented in Table (6) are in partial agreement with the finding of Hilton *et al.* (1980) Hilton and Hodson (1983), who explained no significant differences ($P > 0.05$) detected in hemoglobin or hematocrit levels or red blood cells count of trout fish reared on different diets (0.6, 6.6 and 11.4μg Se/ g dry diet). On the other hand, results obtained by Salte *et al,* (1988) indicated that average hematocrit value (%) increased from 22.1 ± 4.1 (± S.d) to 33.7 ± 2.1 after treatment with 50 mg vitamin E/ kg body weight and selenium at 0.12 mg/ kg body weight of Atlantic salmon diets.

Dimanov *et al.* (1998) found that supplementation with Se and vitamin E separately or together (0.03 to 0.5 mg/g dry diet) increased haemotacrit value in tilapia fingerlings from 18 to 41%.

Total serum protein of the hybrid tilapia fed on diets containing 8 mg Se/ kg supplemented with high vitamin E (300 IU/ kg) was significantly higher ($P < 0.05$) than those fed deficient selenium with low level of vitamin E (100 IU/ kg diet) (Table 6& Fig.II). In contrast, Hilton and Hodson (1983) reported that no significant differences ($P> 0.05$) were detected in plasma protein of rainbow trout reared in different diets supplemented with selenium (0.6, 6.6 and 11.4 μg Se/ g diet). Hilton *et al.* (1980) found that plasma protein levels did not vary significantly ($P > 0.05$) with trout fed diets different in selenium levels from 0 to 15 μg/ g diet, but that of trout fed 15 μg Se/ g diet appeared to be slightly higher than the remaining groups.

e) Liver histopathology:

In the present study, the liver of hybrid tilapia *(O-niloticus X O-aureus)* fed on the 2mg Se with 300IU vitamin E/kg diet was of dull grey-red in color in comparison to the red-brown liver of the other groups fed with selenium supplements ranging between 0,4 and 8 mg Se. These results confirm there of Hilton *et al.,* (1980), who recorded a definite increase in the rate of selenium uptake in the liver when the dietary selenium concentration was above 0.38μg/g. Bell *et al.,* (1985) mentioned that no frank Se-deficiency symptoms have been found in trout *(Salmo gairdneri)* when diets contained an adequate amount of vitamin E.

The prevalence of histological alterations observed in the liver of hybrid tilapia, *(Oreochromis niloticus* X *Oreochromis aureus)* fed on higher percentage of selenium (8mg/kg with low vitamin E,100IU/kg) were much greater than those shown in the same species fed on lower

percentage (without treatment), compared with the control fish fed selenium deficient diet and low vit. E, supplement (100IU/kg diet) as given in Figure (III).

Section in liver of *Oreochromis niloticus* control group showed hepatocytes, kupfler cells, central vein, (C) hepatocytes with eosinophilic cytoplasm with small vacuoles and large central nucleus (E) and hepatocytes with large vacuole and small eccentric nucleus.

Histological examination of the liver sections of the fish species fed on diets containing 4mg Se with 100IU vitamin E/ kg showed degeneration and fibrosis in tissue (Fig. III. a). Hemorrhages and degeneration in hepatic cells were also observed (Fig. III. b). Dilatation in blood vessels and degeneration in surrounding hepatic cells were prominent features (Fig III. c).

The liver sections of hybrid tilapia fed diets with 8mg Se and 100 IU vitamin E /kg showed odema, haemosiderin granules (Fig. III.d) in addition to hemolysis and fatty degeneration (Fig. III.e).

The fatty degenerative changes in *O.niloticus* x *O.aureus* hybrid liver. may be due to the decrease in the rate of utilization of energy reserve or pathologically enhanced synthesis of fat (Desai *et al.,1984*). Also. the abnormal accumulation of fats in experimental animals fed on diets containing 8mg Se with 100IU vitamin E/kg could be due to induced imbalance between fat production and utilization . On the other hand, the liver of hybrid tilapia fed on low supplement of vit. E, (100IU/gk/diet) revealed hemolysis and necrosis around blood vessels and activation in nuclear division (Figs. III. f & g).

The present study suggests a strong link between selenium and lesions in the liver. Hilton *et al.* (1980) recorded that the selenium uptake and accumulation in tissues of the trout *(Salmo gairdneri)* reared on diets containing an excess (3μg Se /g dry feed) may ultimately be toxic to trout if maintained over long periods of time. Also, they found that the storage of excess selenium, particularly in the same species fed on dietary selenium (4.29 and 15.00μg/g diet) after 20 weeks is probably followed by detoxification of selenium by the liver into some methylated derivative or selenoprotein compounds as suggested by the lack of histopathological effects.

In conclusion, results revealed that supplementation of vit E 300 IU/ kg dry diet with selenium up to 2mg / kg dry diet of dietary hybrid tilapia is waranted to increase growth rate and prevent fish from increased concentration of waterborne Se up to toxicity levels.

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FIGURE III

(a1) Section in liver of *Oreochromis niloticus* control: 1) hepatocytes, 2) kupfler cell, 3) central vein, C) hepatocyte with eosinophilic cytoplasm with small vacuoles and large central nucleus E) and hepatocyte with large vacuole and small eccentric nucleus (Hx.& E–X. 400).

(a) Degeneration in wall of blood vessels and hepatic cells (Hx.& E–X. 400). Fibrosis is shown in tissue (Hx.& E–X. 400).

(b) Hemorrhages and degeneration in hepatic cells (Hx.& E–X. 400).

(c) Dilatation and degeneration in blood vessels with degeneration in hepatic cells (Hx.& E–X. 400).

(d) Odema and hemosidren (Hx.& E–X. 400).

(e) Hemolysis and fatty degeneration (Hx. E–X. 400).

(f) Hemolysis and necrosis around blood vessels (Hx.& E–X. 400).

(g) Activation in nuclear division (Hx.& E–X. 400).

Table (1) Treatments and experimental design

	Nutrient Supplement	
	Vitamin E(1) IU/kg diet	**Selenium(2) Mg/kg diet**
1	100 +	0
2	100 +	2
3	100 +	4
4	100 +	8
5	300 ++	0
6	300 ++	2
7	300 ++	4
8	300 ++	8

(1) Vitamin E as Dl - α -tocopherol + low supplemented vit. E
(2) Selenium as sodium selenite ++ high supplemented vit. E

Table (2) Composition, proximate analysis and selenium contents of the basal diet.

Ingredient %	Percent in diet
Fish meal	20
Soybean meal	17
Gluten meal	13
Wheat bran	44
Sunflower oil	3
Vitamin and mineral premix[1]	3
Proximate analysis:	
Moisture	11.52
Crude protein	31.02
Crude lipid	5.93
Crude fiber	6.89
Ash	5.70
Nitrogen free extract	38.94
Metabolizable energy[2] (Kcal/kg)	3240
Selenium content (mg/kg)	0.19
Vitamin E (IU / kg)	46

(1) Vitamin and mineral premix (each 1kg contains: 4-8 IU.Vit. A; 0.8 M.I.U.; Vit. D3: 1500 I.U.Vit.E; 0.8g-Vit-K; 4g. Vit. B12; 4.0g.Vit.B2; 0.6g.Vit.B8; 4.0g.Vit. pantothenioc acid; 8.0g.Vit. Nicotinic acid; 400mg Vit. Folic acid; 20 mg Vit. Biotin; 200g. choline chloride; 4.0g copper; 0.4g Iodine; 12g Iron; 22g Manganese; 22g zinc and 6mg selenium.

(2) The estimated values ME (cited by **Chou and Shaiu, 1996,** for dietary tilapia) used for calculation in this study were : protein, 4.5 Kcal g^{-1} **(Smith, 1971);** Carbohydrate, 3.49 K cal g^{-1} **(Chiou and Ogino, 1975),** Lipid, 8.51 Kcal g^{-1} **(Austreng, 1978).**

Table (3) Growth Performance and mortalities of the hybrid tilapia fed the diets containing different vitamin E and Selenium levels

Parameter	*Nutrient supplement / kg dry diet*							
	Low vit. E (100 IU)				*High vit. E (300 IU)*			
Se Supplement(mg/kg)	*0*	*2*	*4*	*8*	*0*	*2*	*4*	*8*
Mean initial body weight (g)	46.3±1.15 C	48.1±0.75 c	47.3±0.9 b	45.7±1.05 c	46.5±1.15 bc	46.9±1.20 a	46.8±0.95 b	47.0±0.80 b
Mean final body weight (g)	151.4 ±1.88 C	158.3±1.40 c	174.8±1.39 b	152.5±1.83 c	162.2±1.16 bc	188.4±2.05 a	171.1±1.73 b	168.2±1.4 b
Percentage body weight gain (1) (%)	227.0±5.69 C	229.1±3.11 c	269.6±3.15 b	233.7±4.38 c	248.8±2.66 bc	301.7±4.67 a	265.6±3.69 b	257.9±2.89 bc
Specific growth rate (2) (%/day)	0.99±0.02	0.99±0.01	1.09±0.01	1.00±0.02	1.04±0.01	1.16±0.01	1.08±0.02	1.06±0.02
Mortality of number	6	3	3	5	2	2	2	4

a,b and c values in the same row with different superscript letters are significantly different ($P < 0.05$)

* Mean values with their standard errors for thirty fish per rearing unit

(1) Percentage weight gain (WG %) = 100 (Final body weight – Initial body weight) / Initial body weight.

(2) Specific growth rate (%/day) = 100 (Ln final body weight – Ln initial body weight) / days

Table (4) Effect of selenium and vitamin E levels on nutrient utilization of hybrid tilapia

Nutrient supplement/kg diet		*Feed intake (g)*	*Feed Conversion(1)*	*Feed conversion efficiency %(2)*	*Protein efficiency ratio(3)*
VitaminE(IU)	*selenium (gm)*				
Low (100 IU)	0	282.1	$2.68^{a} \pm 0.65$	$37.26^{b} \pm 2.34$	$1.20^{b} \pm 0.11$
100 IU	2	298.3	$2.71^{a} \pm 0.69$	$36.94^{c} \pm 3.09$	$1.19^{b} \pm 0.09$
100 IU	4	309.3	$2.43^{bc} \pm 0.55$	$41.22^{a} \pm 3.15$	$1.33^{a} \pm 0.12$
100 IU	8	277.5	$2.60^{bc} \pm 0.47$	$38.49^{bc} \pm 4.25$	$1.24^{ab} \pm 0.06$
High (300 IU)	0	295.8	$2.56^{b} \pm 0.49$	$39.12^{ab} + 4.25$	$1.26^{ab} \pm 0.10$
300 IU	2	332.0	$2.35^{c} \pm 0.54$	$42.62^{a} \pm 3.74$	$1.37^{a} + 0.07$
300 IU	4	309.1	$2.49^{b} \pm 0.53$	$40.21^{a} \pm 4.21$	$1.30^{a} \pm 0.09$
300 IU	8	299.5	$2.47^{bc} \pm 0.42$	$40.47^{a} \pm 2.16$	$1.30^{a} \pm 0.07$

a, b and c values in the same row with different superscript letters are significantly different ($P < 0.05$).

* Mean values with their standard errors for thirty fish per rearing unit

(1) Feed conversion ratio (FCR) = Total feed consume (g) x100 (final body weight (g) – Initial body weight (g)).

(2) Feed conversion efficiency (FCE)% = Wet weight gain (g)/ total feed consume (g) × 100.

(3) Protein efficiency ratio = Wet weight gain (g)/ protein intake.

Table (5) Effect of Se and Vit. E levels on body composition , energy and protein retention in muscle hybrid tilapia

Supplement		Se and Vit. E Supplement/ kg dry diet							
		Low Vit. E (100 IU)				High Vit. E (300 IU)			
Item	Initial	0	2	4	8	0	2	4	8
Moisture	75.79 ± 1.63	75.15 ± 1.35	74.62 ± 2.01	76.08 ± 1.39	74.89 ±0.96	75.11 ± 1.64	75.32 ±1.75	74.32 ±0.89	75.02 ±1.42
Crude protein	14.15 ±0.49	14.58 ±0.63	14.14 ±0.74	13.69 ±0.96	13.41 ±1.41	13.91 ±0.92	14.15 ±0.85	14.10 ±0.67	13.76 ±0.72
Crude lipid	6.47 ±0.25	5.82 ±0.57	6.21 ±0.64	5.81 ±0.82	5.96 ±0.57	6.05 ±0.63	5.69 ±0.51	6.21 ±0.60	6.15 ±0.56
Ash	3.51 ±0.13	4.41 ±0..14	5.03 ±0.24	4.39 ±0.16	5.72 ±0.20	4.93 ±0.19	4.80 ±0.27	5.36 ±0.21	5.07 ±0.24
Gross energy(1) (kcal/100g)	142.10 ±5.27	138.4 ±4.09	139.6 ±5.21	133.23 ±4.73	133.1 ±4.70	136.76 ±5.57	134.7 ±3.95	139.4 ±4.17	136.9 ±5.96
Protein (2) retained (%)	--	17.74^{ab} ±1.56	16.84^{b} ±0.96	17.97^{a} ±1.24	16.25^{b} ±1.73	17.42^{ab} ±2.10	19.44^{a} ±1.86	18.26^{a} ±1.93	17.75^{ab} ±2.14
Energy (3) retained	--	15.73^{b} ±1.67	15.79^{b} ±1.45	16.55^{ab} ±2.01	15.35^{b} ±1.43	16.25^{ab} ±2.11	17.40^{a} ±2.43	17.18^{a} ±1.96	16.85^{a} ±2.01

a and b values in the same row with different superscript letters are significantly different ($P < 0.05$).

(1) Muscle gross energy (Kcal/ 100g muscle) by NRC (1983) = 5.7 × % crude protein + 9.5 × % ether extract.

(2) Protein retained % (PR) by Chou and Shiau (1996) = 100 × (Final body wt. × final body protein – Initial body wt. × Initial body protein). Total feed intake x dietary protein.

(3) Energy retained % (ER) by Chou and Shiau (1996) = 100 × (Final body wt. x final body energy – Initial body wt. x initial body energy). Total feed intake x dietary energy.

Table (6) Hepatosomatic index, hematocrit, hemoglobin and total serum protein in hybrid tilapia as affected with the experimental diets

Nutrient supplement (1kg/diet)			Hepatosomatic Index (%)	Hematocrit value (%)	Hemoglobin concentration g/100 ml	Total serum protein g/ 100 ml
Vitamin E (IU)		Selenium				
Low	(100 IU)	0	$1.55^{bc} \pm 0.05$	$28.34^{b} \pm 1.92$	6.82 ± 1.31	$4.07^{b} \pm 0.52$
	(100 IU)	2	$1.76^{a} \pm 0.04$	$31.20^{ab} \pm 2.12$	6.96 ± 0.95	$5.18^{a} \pm 0.39$
	(100 IU)	4	$1.95^{a} \pm 0.07$	$32.12^{ab} \pm 1.99$	7.25 ± 1.04	$5.49^{a} \pm 0.56$
	(100 IU)	8	$1.52^{c} \pm 0.06$	$33.94^{a} \pm 1.24$	7.63 ± 1.12	$5.64^{a} \pm 0.42$
High	(300 IU)	0	$1.70^{ab} \pm 0.05$	$30.18^{ab} \pm 1.65$	7.79 ± 0.86	$4.42^{ab} \pm 0.069$
	(300 IU)	2	$1.63^{bc} \pm 0.05$	$33.28^{a} \pm 1.16$	7.53 ± 0.99	$4.56^{ab} \pm 0.32$
	(300 IU)	4	$1.48^{c} \pm 0.04$	$31.40^{ab} \pm 1.40$	7.26 ± 1.14	$4.95^{ab} \pm 0.47$
	(300 IU)	8	$1.80^{a} \pm 0.06$	$34.64^{a} \pm 0.96$	6.95 ± 1.08	$5.63^{a} \pm 0.51$

a , b and c values in the same row with different superscript letters are significantly different ($P < 0.05$).

* Mean values with their standard errors for ten fish per treatment

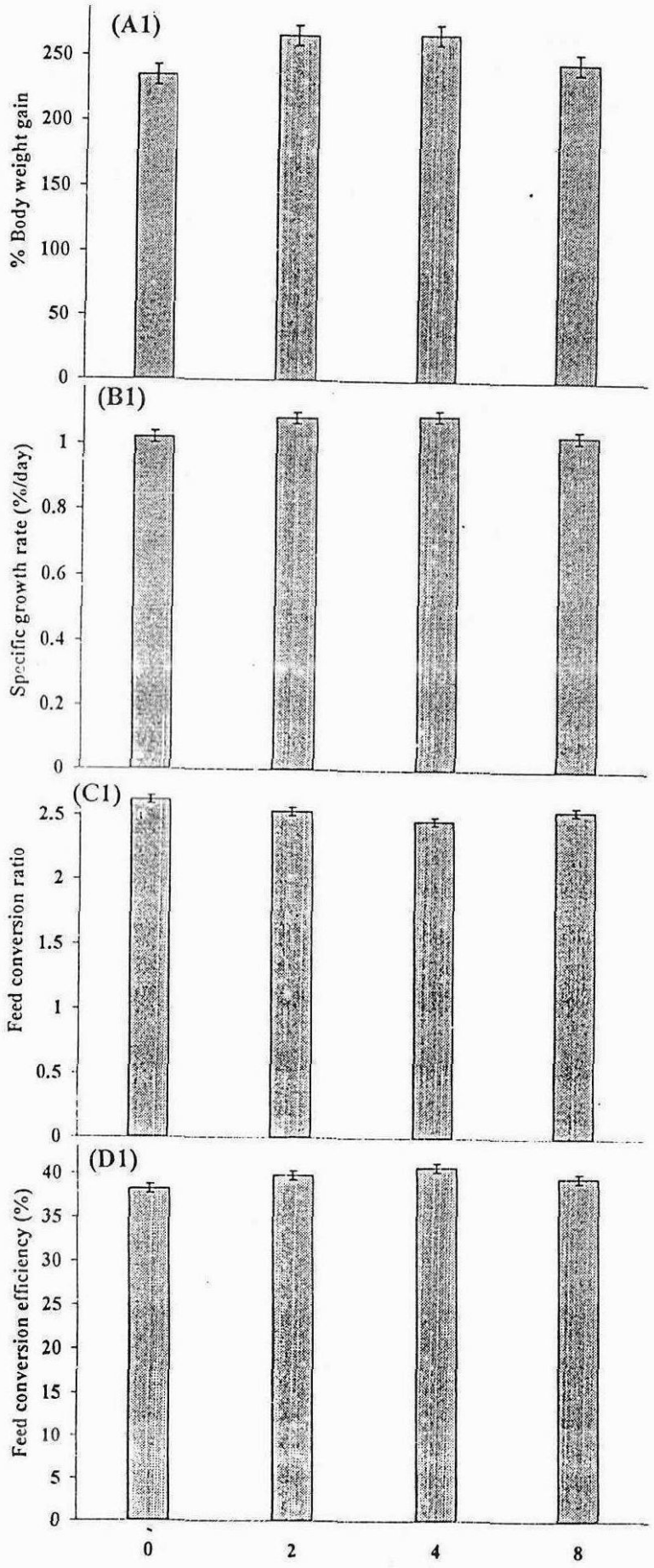

Fig. 1(A, B, C & D) : Percentage body weight gain, specific growth rate, feed conversion ratio and feed conversion efficiency of hybrid tilapia fed different level of supplemented selenium irrespective of level vitamin E.

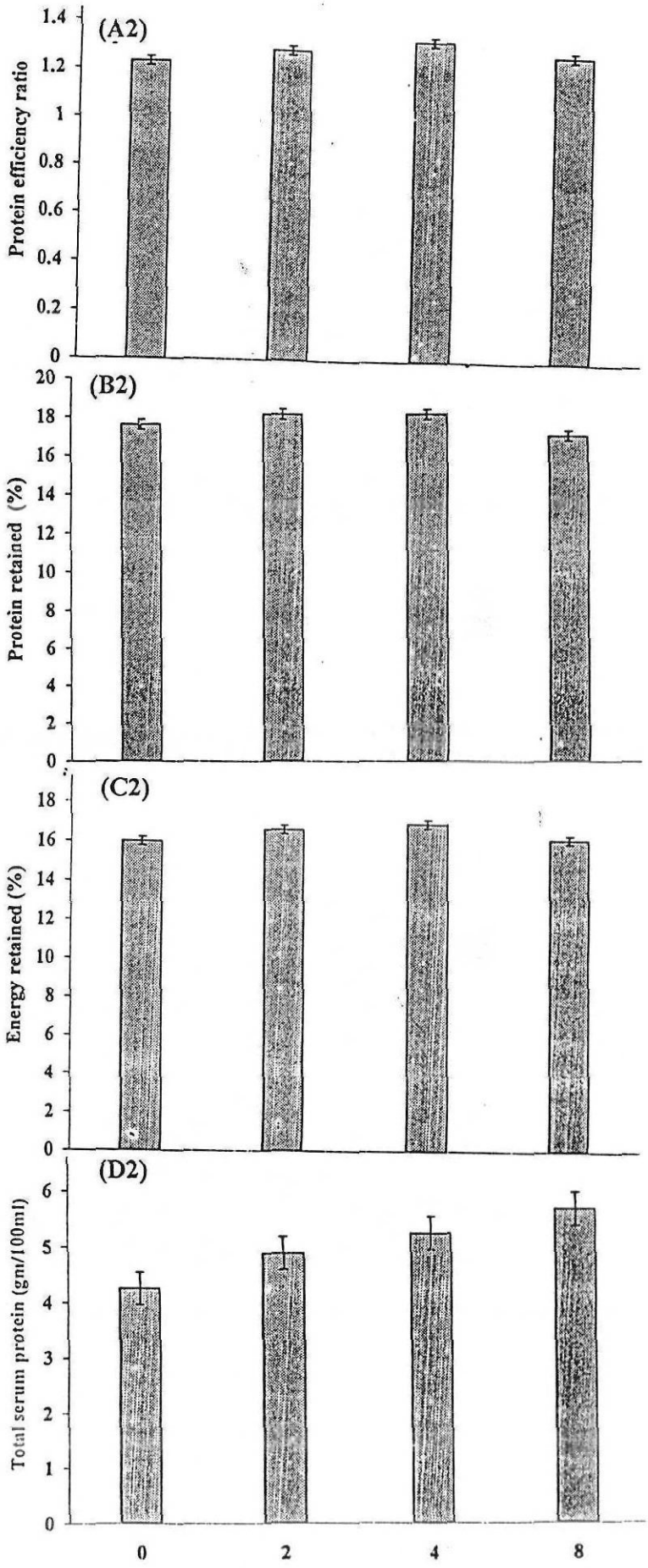

Fig. 2(A, B, C & D) : The effect of supplemented selenium level irrespective of vitamin E level on feed utilizatio efficiency and total serum protein of hybrid tilapia.

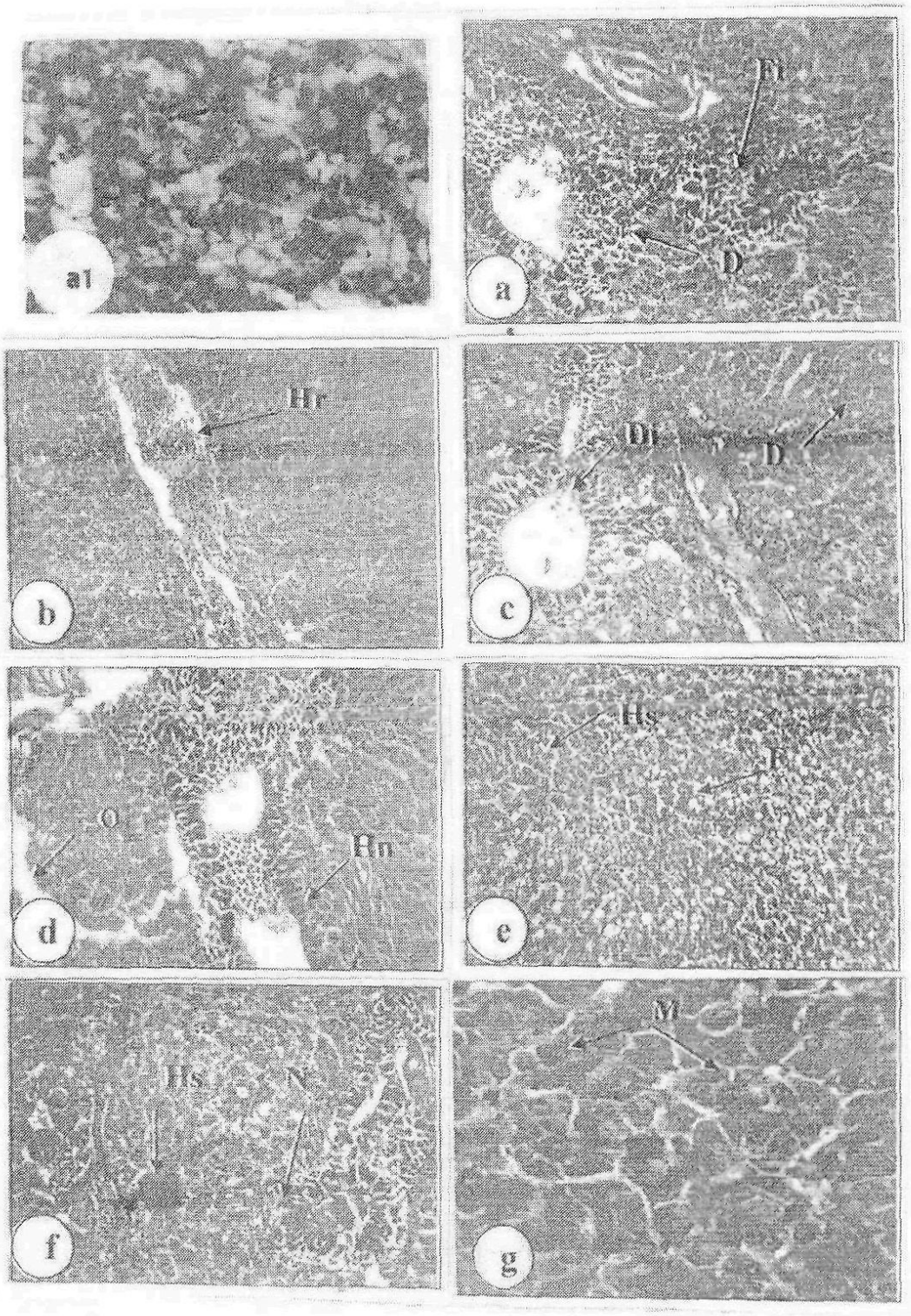

Figure III