INTRODUCTION AND AIM OF THE WORK

Fish production has been considered as one of the main sources of animal protein in many developing countries including Egypt. Owing to the apparent deficiency in animal protein, increasing fish production has been considered, in the last few years, as one of the main goals of the food security program in the world. Fish culture allover the world has been improved in order to increase the quality and quantity of different types of fish (Haggag *et al.*, 1977). Hepher and Preginin (1981) showed that, the aquaculture is based on existing water bodies rather than on ponds constructed specially for fish culture.

Therefore, the development of fish culture can be achieved by increasing of rearing fish in ponds and fish farms (Ishak, 1985). The potential of fish farming in Egyptian fresh water ponds is so great due to the suitable climate, availability of cultured fish fry, availability of water and fertility of the lands (Hassanen, 1986). Similarly, Beveridge (1987) mentioned that, the aquaculture or the farming of aquatic organisms is achieved through the increasing of plankton organisms (phyto and zoo plankton) and control of the environmental factors (physico-chemical properties) which influence it. Consequently several studies were conducted in different countries to improve the growth rate and reproduction of fish species .This improvement can be achieved through using suitable dietary

protein level (Hassanen 1986), control of environmental factors (Beveridge, 1987), addition of certain chemical compounds to the fish diets (Siddiqui *et al.,* 1988) and increasing energy contents in artificial diets (Degani *et al.,* 1989).

Food is considered the most potent exogenous factor affecting the growth rate and sexual maturity of many fish species (Alexis *et al.,* 1986). Practical experience has shown that Nile tilapia **(*Oreochromis niloticus*)** can readily accept artificial feeds of varying quality at all life stages from the first feeding of the fry (Santiago *et al.,* 1987). This is one characteristic that makes it a favored species for high density culture to give maximum fish production (Al-Zahaby *et al.,* 1987).

Many compounds such as hormones, vitamins and mineral salts (Dabrowski *et al.,* 1993) were added to the fish food to increase metabolic functions and biochemical reactions in fish bodies. Vitamins are very important nutrients in the supplementary diets of most fish species (Dabrowski and Blom, 1994). As they are considered as important food factor and the absence or relative deficiency of the vitamins in the diets leads to decrease fish appetites, metabolic activities and consequently to the diseases (Abdelghany,1998). They are also called accessory food factors.

Vitamin C (L-Ascorbic acid) has a highly efficient role in growth rate and reproduction of different fish species (Wilson,

1973). It is also essential for normal metabolism of amino acids, lipids and minerals (Lehninger,1982). The fish depend upon an exogenous source of vitamin C, as they cannot synthesize it.

The histochemical and biochemical studies of Soliman *et al*. (1986) showed that the absence of the enzyme L-gulonolactone oxidase in the liver and kidney of several species of tilapia is due to the conversion of the enzyme to ascorbic acid (Dabrowski,1990).Blom and Dabrowski (1993) reported that, the tissues vary considerably in their concentration of ascorbic acid and that the gonad represents one of the organs containing highest level of it. Therefore, Dobrowska & Ciereszko (2001) mentioned that, ascorbic acid is an essential compound in the regulation of the endocrine functions and gamete quality (fertility) in most fish species.Vitamin E (α-tocopherol) is also an essential micronutrient in fish culture to increase the growth performance and other biological activities (Ronnested *et al.*, 1999). Vitamin E is a fat soluble intracellular antioxidant involved in stabilizing unsaturated fatty acids. Idris & Hassan (2002), mentioned that vit.E increases the resistance of RBCs membranes to rupture and hemolysis caused by oxidizing substances (eg.H_2O_2). On the other hand, vit.E is called anti-sterility vitamin, thus it is important for the improvement of the fertility of the gonads (Martin *et al.*, 1981). Takeuchi *et al.*, (1981) reported that, impaired fertility, hatchability and fry survival were observed in eggs originating

from females of fishes provided with diets containing α-tocopherol (vitamin E).

Cobalt salts in the form of cobalt chloride and cobalt nitrate have an important role in fish culture systems for improvement of fish production. Recently, Hamza and Kenawy (1997) studied the effects of cobalt chloride and cobalt nitrate in the artificial diets on growth rate of mullet fish (***Mugil cephalus*** and ***Liza ramada)***. They noticed that, the survival rate and growth of fish have been increased with cobalt chloride than cobalt nitrate compared with fish fed without cobalt salts (control group).

The selection of the Nile tilapia, ***Oreochromis niloticus*** for the present study is due to its important role in the Egyptian fisheries since it provides more than 70% of the total fish production in Egypt (Ishak *et al.*, 1985). On the other hand, Santiago *et al.*, (1987) and Siddiqui *et al*. (1988) mentioned that, the culture of Nile tilapia, ***O. niloticus*** depends mainly on the artificial feeds in its rearing at all life stages from the fry to the adult. Therefore, tilapia constitute the third largest group in fish culture in different countries after carps and salmonids (EL-Sayed, 1999). These fish species were also ideally suitable for different culture systems because they are disease-resistant, reproduce easily and can tolerate unfavorable conditions of water (Attalla, 2001).

Aim of the work

The present study is conducted to demonstrate the effect of vitamins C (ascorbic acid), E (α-tocopherol) and $CoCl_2$ (cobalt chloride) separately and in combination with each other on the biological conditions and fecundity of Nile tilapia, ***Oreochromis niloticus*.** Also, to illustrate the best treatment that gives the maximum growth rate and optimum feed utilization as compared with an untreated group (fish fed normal diets without any additions). Moreover, this study clarifies the influence of the previous micronutrients on the biochemical analysis of liver, muscles and gonads of the studied fish, ***O. niloticus*.** In addition, it clarifies the role of these treatments in alleviation of pollution effects on some tissues (liver, ovary and testes) of the studied fish**.**

REVIEW OF THE LITERATURE

Nile tilapia (***O.niloticus***) is one of the most important species of family Cichlidae. About 700 species of this family are naturally distributed throughout the world of which 80 species are referred to have the common name tilapia but only eight species feature significantly in aquaculture (Pullin, 1983). Tilapia culture has been expanded in different areas for its potential for production as food fish, forage fish, bait fish and for control of aquatic weeds. Broussard (1985) reported that, the scientific name of Nile tilapia was firstly ***Tilapia nilotica*** (Prior) to the year 1980, ***Sarotherodon niloticus*** (Artedi) from 1980 to 1982 and ***Oreochromis niloticus*** (Linnaeus) thereafter. El-Sayed (1999) mentioned that, tilapia species become important group in different systems of fish culture for their rapid growth, high tolerance to low water quality, efficient feed conversion, ease of spawning, resistance to disease and good consumer acceptance.

1. Physico-chemical properties of tilapia ponds:

Tilapia have been adjusted to particular environments and obviously for each species there will be an upper and a lower limits for all physical and chemical parameters of the environment. Temperature, dissolved oxygen, alkalinity, nitrate, nitrite, pH value and ammonia are the major Physico-chemical factors to be considered and influence when tilapia species are

evaluated to their suitability for culture (Boyd,1979 and Shehata *et al.*, 1995).

1.1. Water temperature

The control of water quality of fish ponds is considered the key of success for fish farming production and profitability. Water temperature is an important factor of water quality in tilapia ponds, most tilapia do not eat or grow at water temperature below 15°C (Bradach *et al.*, 1972) and do not spawn at temperature below 20°C (Rothbard, 1979 and Chervinski,1982). Meske (1985) mentioned that, the water temperature must be kept above 24°C for optimal growth and production of tilapia species. Rakocy and McGinty (1989) conferred that, the preferable water temperature for optimum tilapia growth is 28-30ºC. Whereas, at temperatures below 12°c tilapia lose their resistance to disease and death will occur below 10°C. Eid and El-Danasoury (1991) mentioned that, increasing water temperature from 16 to 27°C improved the growth rate, protein efficiency ratio and decreased the mortality rate of Nile tilapia, ***O. niloticus***. Watanabe *et al*. (1996) found that, the final body weight, feed conversion ratio and protein efficiency ratio had their optimum values at water temperature ranging from 26 to 32°C.

1.2. Dissolved oxygen

Broussard (1985) pointed out that, tilapia can tolerate extremely low levels of dissolved oxygen in fish ponds, while

the growth rate is reduced at short periods of low oxygen. The same author added that, tilapia need about 5mg /L of oxygen or more to avoid stressful conditions. Sweilum (1995) cited that, the oxygen content for best growth and reproduction of Nile tilapia, ***O. niloticus*** reared in earthen ponds ranged from 8.0 to 8.3 mg/L.

1.3. pH value

Boyd (1979), Fromm (1980), Johnson (1986) and Teichert-Coddington and Green (1993) reported that, waters with pH ranging from 6.5 to 9 are most suitable for fish growth, production, reproduction and survival rate.

1.4. Total alkalinity

The alkalinity of ponds does not have a direct effect on tilapia as much as an indirect relationship to pond productivity (Huet, 1970). Osmotic stress due to high alkalinity may promote the onset of thermal stress and anoxia (Morgan, 1972). However, tilapia are generally believed to have a high tolerance limit for alkalinity. The same author showed that, the inorganic carbonate was presented in ponds water as a mixture of free CO_2 with a very small amount of H_2O_3 and CO_3^{-2}. Siliem (1995) indicated that, the alkaline natural water seems to be more productive than acid water and waters of low alkalinity are generally biologically less productive than those with high value.

1.5. Nitrate and nitrite

Abdel-Hamid *et al*. (1992) reported that, raising of nitrate (NO_3) in water at fish ponds is generally due to the discharge of drainage water which carried nitrate fertilizers previously used for vegetables, grass and many crops.

1.6. Total ammonia

Broussard (1985) and Johnson (1986) indicated that, ammonia is toxic to tilapia in rearing ponds at a level of 1-2 mg / L. However, Johnson (1986) added that, losses begin at more than 5mg/L. Ammonia toxicity is related to pH as the unionized form of ammonia prevalent at high pH is toxic and tilapia can tolerate high ammonia levels than other fish species. Teichert-Coddington *et al.* (1992) found that, supplemental nitrogen can increase fish yields, but total ammonia exceeding 0.15mg / L will not result in higher fish yield.

2. Supplementary diets

Protein is the principal constituent of fish tissues and organs, thus a liberal and continuous dietary protein supply is required for growth and production in rearing tilapia species. Sweilum (1995) and AL-Hafedh (1999) mentioned that, the level of dietary protein that produced maximum fish growth and production ranged from 28 to 30%. Also many substances were added to the fish food for increasing the growth rate and production of reared fish among which are vitamins (Ronnested *et al.,* 1999 and EL-Hammady, 2002), hormones (Lone and

Matty, 1983) and minerals (Kheir and saad, 2002). In addition other growth promoting materials such as cobalt (Hamza and Kenawy, 1997), chromium (Hertz *et al.,* 1989), astaxanthin (Reholkay, 2000) and L-carnitine (Beckar *et al.,* 1999) are added as salts to fish diets.

2.1. Vitamins:

Vitamins are organic compounds required in the diet in relatively small quantities for growth, health and physiological functions in animals. The quantitative requirements of vitamins are based on the minimum level that will support maximum growth of the fish without deficiency symptoms or mortality. Vitamins are classified as water soluble and fat soluble vitamins. Eight of the water soluble vitamins are required in relatively small quantities and have primarily coenzyme functions (e.g. B complex). Three other water soluble vitamins (vitamin C, inositol and choline) are required in large quantities and have other functions. Whereas the fat-soluble vitamins are A, D, E and K (Lovell, 1989). Watanabe (1995) mentioned that, vitamin requirements for fish vary with species, size and environmental conditions.

2.1.1. Vitamin C

Vitamin C is L-ascorbic acid and acts as cofactor for many enzymes in animal (fish) bodies and is important for formation of bones, cartilage and blood capillaries.

The fish depend upon exogenous source of vitamin C as they cannot synthesize it, due to the lack of the enzyme L-gluconolactone oxidase which catalyses the terminal step in the conversion of glucose to ascorbic acid (Chatterjee, 1978). Requirement of vitamin C for rearing fish varies with fish species, fish size and environmental conditions of fish ponds. The National Research Council, NRC (1991) indicated that, vitamin C requirement for tilapia species is 50µ / Kg. diet. Whereas, Shiau and Jan (1992) suggested that, vitamin C requirement for maximum growth of tilapia fish is 79 mg / kg diet. Abdelghany (1998) reported that, increasing the level of vitamin C to 50 mg/kg diet for the Nile tilapia, ***O. niloticus*** gives the best growth and feed utilization. However, Shiau and Hsu (1999) found that, 70 mg / Kg diet of vitamin C gave significantly more body weight in hybrid tilapia (***O. niloticus x O. aureus***). While, EL-Hammady and EL-Sayed (2002) noticed that, the diet supplemented with 400 mg/Kg diet of vitamin C gave higher weight gain and better feed conversion ratio in tilapia hybrid (***O. niloticus x O. aureus***).

2.1.2. Vitamin E

Vitamin E is also called tocopherol which is the methyl derivative of tocol α-tocopherol. It has the greatest biologic activity and is the most active form of vitamin E. Vitamin E has an antioxidant property, i.e. it protects the carotenoids from destruction by free radicals and inhibits damage of lung tissue by oxidants. Vitamin E increases the resistance of RBCs to

rupture and hemolysis by oxidizing substances (e.g. H_2O_2). So vitamin E deficiency causes anemia where it protects the membrane of the red blood cells from destruction. In all animals including fish, vitamin E is important for fertility so it is also called anti-sterility and anti-dystrophic vitamin. Therefore, Sandnes *et al*. (1984) reported that, vitamin E has a specific role in reproduction of most teleosts (fish species). NRC (1993) mentioned that vitamin E requirement for tilapia is so 1µ/kg diet. Abdel-Hamid *et al*. (1999) found that, vitamin E has a significant effect on growth performance and feed utilization of Nile tilapia (***O. niloticus)***. Abdel-Kafy (2000) mentioned that, treatment of rabbits with vitamin E improved the ovulation rate by 64% than untreated rabbits. Gimenez *et al.* (2004) indicated that, the dietary levels of vitamin E was highly effective on growth, survival and hepatopancreas of shrimp.

2.1.3. Cobalt salts

Investigations for importance of fish survival and growth by addition of cobalt salts (cobalt chloride and cobalt nitrate) to their feed were studied by several investigators. Roginski and Mertz (1977) reported that, the cobalt binds to insulin in animals and affects the growth and hematological characters of several fish species. Hertz *et al.* (1989) studied the effects of cobalt and chromium on glucose metabolism in the common carp (***Cyprinus carpio***) and noticed that, treatment with cobalt salts improved glucose utilization and inhibited gluconeogenesis. The authors added that, the addition of cobalt

chloride to tilapia diets leads to better growth and optimum feed utilization. Hamza and Kenawy (1997) added cobalt chloride and cobalt nitrate to diets of ***Mugil cephalus*** and ***Liza ramada*** and found that, these salts could improve fish culture management operations during the critical nursing stages.

3. Growth rate:

The growth rate (performance) of reared fish was indicated by determining the growth in length and weight, specific growth rate and percentage weight gain (Sweilum, 1995; Hassouna *et al.*, 1998 and EL-Shandweily, 1999).

3.1. Growth in length and weight

The growth in length and weight of tilapia species is affected by several factors as stocking density, dietary protein level, feeding rate, feeding frequency, feeding addition, growth promoters, fertilizers and water quality of rearing ponds. Sweilum (1997a) reported that, the best growth in length and weight of Nile tilapia, ***O. niloticus*** was obtained from fish fed on 30% dietary protein level. Hassouna *et al.* (1998) found that the total length and body weight of ***O. niloticus*** had their highest values when fish ponds were fertilized with urea and super phosphate. Abdel-Hakim and Moustafa (2000) mentioned that, the Nile tilapia, ***O. niloticus***, exhibited very good performance when stocked at a rate of 80 to 120 fish /m^3 and fed on commercial diet containing 28-32% crude protein. Sweilum (1997b) showed that, the growth of fish size (length

and weight) was increased with increasing of feeding rate to 5% of fish weight and feeding frequency was twice per day. El-Shandweily (1999) noticed that the physico-chemical properties of water in fish ponds were highly effective on growth of total length and body weight of reared fish. Ali (2001) mentioned that the addition of vitamin E and biogen to diets of Nile tilapia, ***O. niloticus***, leads to improvement of growth in length and weight of the fish. Salah EL-Din (2004) fed the Nile tilapia on artificial diet containing different levels of protein-energy and noticed that, the highest growth in length and weight of fish were observed at 30% protein with 3000 Kcal.

3.2. Specific growth rate

The specific growth rate is the relation between weight gain of reared fish and rearing period. It varies with different experimental treatments. Sweilum (1998) indicated that, the Nile tilapia, ***O. niloticus***, need 30% dietary protein for giving highest specific growth rate (SGR). AL-Hafedh (1999) reported that, the SGR of ***O. niloticus*** increased to 1.07% with increasing the dietary protein level to 45%. However, Sweilum (1997b) mentioned that, the SGR of ***O. niloticus*** reached its maximum value in fish fed at a feeding rate of 5% body weight. Siddiqui *et al.* (1988) found that, the highest specific growth rate of ***O. niloticus*** was obtained with a diet containing 30% protein for young stage and 40% protein for fry stage. EL-Hammady and EL-Sayed (2002) cited that the maximum SGR (1.13% day) of hybrid tilapia (***O. niloticus*** x ***O. aureus***) was

observed with a diet containing 400 mg /kg vitamin C (ascorbic acid). Ogunji and Wirth (2000) reported that, the SGR of Nil tilapia increased with increasing the protein level in diets from 7.3 to 44.2%. Abdel-Hakim *et al.* (2001) found that, the SGR for Nile tilapia, ***O. niloticus*** was improved when the dietary protein increased from 25 to 30%. Samra (2002) reported that, the SGR of Nile tilapia was significantly decreased with increasing the dietary energy from 3000 to 3600 kcal /kg.

3.3. Percentage weight gain

Percentage weight gain (PWG) is the relation between the weight gain of reared fish and its initial weight and is also influenced by different factors. Wee and Tuan (1988) indicated that, the percentage weight gain of Nile tilapia had its highest value when the dietary protein level increased from 22 to 25%. While, Sweilum (1997a) mentioned that the PWG of ***O. niloticus*** reached its maximum value at 30% dietary protein level. AL-Hafedh (1999) found that the PWG of Nile tilapia was gradually increased with increasing the dietary protein level until 45%. EL-Hammady and EL-Sayed (2002) reported that the highest value of PWG of hybrid tilapia (***O. niloticus*** X ***O. aureus***) was obtained with feeding on 400 mg / kg. diet vitamin C. On the other hand Samra (2002) concluded that, Nile tilapia, ***O. niloticus***, fed a diet with energy level of 3000 kcal / kg showed the best PWG and increasing the energy level to 3600 kcal / kg diet leads to decreasing of PWG of the reared fish.