

CHAPTER SIX

SUMMARY AND CONCLUSION

The present study was carried out at the Fish Nutrition Laboratory, Department of animal and fish production, Faculty of Agriculture (Saba-Basha) and Reproductive Toxicity Laboratory, Department of Environmental Studies, Institute of Graduate Studies and Research, Alexandria University. This study comprises of two experiments to investigate the following:

- 1- The effects of supplementation of some dietary natural phytochemicals on growth performance, anabolic steroid hormone levels, antioxidant enzymes and physiological response of Nile tilapia, *Oreochromis niloticus*, fingerlings.
- 2- The effects of supplementation of some dietary natural phytochemicals in comparison with 17 α -methyl testosterone (MT) on sex reversal, growth performance, survival, feed and protein utilization of Nile tilapia, *O. niloticus*, fry.

The 1st experiment

Two levels of each phytochemicals supplementations (ginseng extract, tribulus extract and date palm pollen) were compared with control on growth performance, anabolic steroid hormone levels, oxidative stress, antioxidant enzymes, and hematological, plasma biochemical and histological changes; feed utilization and body composition of Nile tilapia (*O. niloticus*) fingerlings for 84 days. Seven treatments applied at 14 aquaria, as follow: 1-control (basal diet containing 32% crude protein, BD); 2- BD supplemented with ginseng extract (0.2 g/kg diet); 3- BD supplemented with ginseng extract (0.4 g/kg diet); 4- BD supplemented with ginseng extract (0.2 g/kg diet) tribulus extract (0.625 g/kg diet); 5- BD supplemented with tribulus extract (1.25 g/kg diet); 6- BD supplemented with date palm pollen (3g/kg diet) and 7- BD supplemented with date palm pollen (6 g/kg diet).

The experimental treatments were carried out in glass aquaria with dimensions of 100×40×30 cm, each aquarium was stocked with 10 fish (3.67±0.02 g/fish) in two replicates. Fish was fed two times a day (9.00 -14.00 hrs) six days a week, at a decreasing rate of 6 to 5 to 4% of the actual live fish body weight every four weeks. Average of water temperature ranged between 26±2°C. Continuous aeration was maintained in each aquarium using an electric air blower. Manual method for removal of excreta was conducted every day before the first feeding by siphoning half of the water volume in each aquarium and replaced by an equal volume of fresh water.

The results of the 1th experiment summarized as follows:

- 1- Dietary supplementation with all studied phytochemicals (Ginseng extract, tribulus extract, date palm pollen) significantly increased growth performance of *O. niloticus* in dose dependent manner. The highest growth performance recorded with high level of tribulus extract. Treatment with all phytochemicals maintain the normal survival rate of fingerlings as compared to control group. Also, fish condition factor improved with treatments.
- 2- Feed utilization values (feed intake, feed conversion ratio, FCR , protein utilization and energy utilization) were improved in *O. niloticus* with all investigated dietary phytochemicals supplementation treatments especially with the high levels,
- 3- The dietary phytochemicals supplementations were slightly increased the *O. niloticus* carcass protein content, however, decrease ether extract content as compared with the control group.
- 4- Treatment with phytochemicals significantly decreased visceral somatic index and increased liver, testes and spleen somatic indexes compared to control.
- 5- Luteinizing hormone and testosterone levels increased significantly in fish received supplemented diet with high level of ginseng extract, tribulus extract and date palm pollen. Moreover, the improvement of growth performance was concomitantly to the increase of testosterone level (anabolic steroids).
- 6- The histological study of fish testes showed that all dietary phytochemicals treatment showed slightly to highly improvement of spermatids abundance. The best histological structure and spermatid abundance illustrated with tribulus extract.
- 7- Treatment with all levels of phytochemicals significantly reduced plasma, liver and testes thiobarbituric acid-reactive substances (TBARs) levels and significantly increased reduced glutathione content (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx) activities than control.
- 8- Fish blood picture results showed that red blood cells (RBCs), haemoglobin (Hb), hematocrit (Hct) and white blood cells (WBCs) significantly increased with high level of each tested phytochemicals compared to control. However, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) didn't show any significant differences with any studied levels.

- 9- The results showed a significant increase in total protein, globulin and high density lipoprotein-cholesterol (HDL-c) with all tested phytochemicals levels compared to control. However, total lipids (TL), cholesterol, triglycerides (TG), low density lipoprotein-cholesterol (LDL-c) and very low density lipoprotein-cholesterol (VLDL-c) significantly decreased with all tested phytochemicals levels compared to control. The best protein and lipids profile showed with tribulus extract (1.2 g/kg diet) followed by ginseng extract (0.4 g/kg diet).
- 10- Ginseng extract, tribulus extract and date palm pollen treated fish showed significantly decrease in plasma urea, creatinine, bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and acid phosphatase (ACP). Meanwhile, in the liver homogenate of treated fish the activities of AST, ALT, ALP and ACP were significantly increased in level dependent manner with different phytochemicals treatments compared to control.

It could be concluded that dietary phytochemical (ginseng extract, tribulus extract, date palm pollen) improved growth performance, anabolic steroid hormone levels, antioxidant enzymes activities, glutathione content and reduced TBARs in plasma, liver and testes of *O. niloticus* fingerlings. Also, it improved liver and kidney function, hematological parameters, feed and nutrients utilization of treated fish. The best results obtained with tribulus extract at high level (1.2 g/kg diet) followed by ginseng extract at high level (0.4 g/kg diet). There is a positive relationship between the increase of endogenous anabolic steroid hormone (testosterone) level which induced by phytochemicals and growth of Nile tilapia fingerlings.

The 2nd experiment:

Two levels of each dietary natural phytochemicals supplementations (ginseng extract, tribulus extract and date palm pollen) and 17 α -methyl testosterone (MT) were compared with control on sex reversal, growth performance, survival, feed and protein utilization of Nile tilapia, *O. niloticus*, fry. Eight treatments were applied as follow: 1- control (basal diet containing 45% crude protein, BD); 2- BD supplemented with 17 α -methyl testosterone; 3- BD supplemented with ginseng extract (0.2 g/kg diet); 4- BD supplemented with ginseng extract (0.4 g/kg diet); 5- tribulus extract (0.625 g/kg diet); 6- BD supplemented with tribulus extract (1.25 g/kg diet), 7- BD supplemented with date palm pollen (3g/kg diet) and 8- BD supplemented with date palm pollen (6 g/kg diet). The treatments received experimental diets for 28 days (end of the experiment) and completed with basal diet until 84 days of the age (to be suitable for dissection).

Nile tilapia fry one day old (0.02 g/fish and 1.13 cm/fish) kept in glass aquaria for one day after transportation without feeding. Fry divided on 16 glass aquaria (100×40×30 cm) two replicate for each treatment with initial density 50 fry/aquarium. Fish reared under the same condition of the first experiment.

The results of the 2nd experiment summarized as follows:

- 1- The effect of dietary supplementation of the tested phytochemicals (ginseng extract, tribulus extract and date palm pollen) on sex reversal observed with tribulus extract (1.2 g/kg diet) were male percent was two third of populations. However, MT treatment recorded the highest male ratio (92.30%) compared to control group (48.36 %).
- 2- The dietary supplementation of the tested phytochemicals in high level significantly improved growth performance than control and MT treated fry, the highest FW was recorded by tribulus extract (1.2 g/kg diet). The total length, length gain and condition factor didn't change significantly compared to control. Moreover, normal survival rate were mentioned.
- 3- The dietary phytochemicals treatments didn't change the palatability of the experimental diets were feed intake and protein intake didn't change significantly. However, FCR and protein efficiency ratio were improved significantly with high levels of all phytochemicals supplementations treatments than control.

Finally, it could be concluded that the used phytochemicals didn't affect the sex ratio of Nile tilapia fry compared with 17 α -methyl testosterone, except the high level of tribulus extract (caused 17% males more than control). All used phytochemicals supplementation improved growth performance, feed and nutrients utilizations of Nile tilapia fry. The best results were obtained with the high levels.

Recommendation:

It is recommended to use tribulus extract at level of 1.2 g/kg diet in Nile tilapia fingerlings and fry to improve the growth performance. Dietary phytochemicals (ginseng extract, tribulus extract and date palm pollen) could be used as growth promoters, but not as masculinization agents in Nile tilapia fry. However, further investigation will be required to determine the optimum tribulus extract level for induction of 100% sex reversal