CHAPTER TWO

REVIEW OF LITERATURE

Food production is without a doubt, a very relevant aspect in human life. Among the different food production activities the most important are agriculture, livestock production and fisheries. These three economic activities and more specifically fisheries, both inland and marine fisheries are close to reaching their maximum capacity in term of sustainable production. In order to reduce the pressure on wild stocks of many fish under commercial use, aquaculture is a viable alternative to fisheries throughout the world (Marra, 2005).

The world aquaculture production duplicated in the last ten years (2001 to 2011) from 34.6 to 62.7 million tones, and contributed 40.1% of the total world fish production (FAO, 2011).Nile tilapia, *Oreochromis niloticus,* is among the species extremely suitable for aquaculture.

Muir *et al.,* (2000) indicates that even considering that the potential of tilapia for intensive production has been realized in general, the species has not had the same immediate appeal that other food fish species. It still provides many desired characteristics. Fitzsimmons, (2000) reviews these characteristics in terms of the unique mix of the tilapia's physiology, reproductive biology, genetic plasticity, development of domesticated strains, and ready marketability. Moreover, the future convergence of improved culture techniques, new farms, low cost diets, ecological efficiency and emerging markets will boost tilapia to be the world largest aquaculture crop. As a final remark this author states that tilapia is likely to be the most important of all aquaculture fish in the 21st century.

Despite promising expectations in tilapia aquaculture, there are a series of factors that are considered to be detrimental in the expansion of production. One of these factors is strong environmental concerns, which have been outlined by several authors in relation to the ecological burden of tilapia aquaculture. These include the amount of organic matter and wastewater discharged due to the fertilization of ponds and use of supplemental feeding to increase production (Yi *et al.*, 2002).

Among the new approaches to integrate tilapia production and wastewater treatment management are those that prove that tilapia can be a useful species in other aspects, not related to food production. Several studies draw attention to the possibility of using tilapia as 'biofilters' in polyculture system with other fish species such as Chinese carps (Papoutsoglou *et al.*, 2001; Abdelghany and Ahmad 2002). Recently, tilapia rearing technology developed to "zero water exchange systems" under the technology of biofloc to remove inorganic nitrogen from wastewater (Avnimelech and Kochba 2009).

Secondly, the release of hormones used to produce monosex populations for intensive aquaculture (Abucay and Mair 1997; Gale *et al.*, 1999). Where a major drawback in tilapia aquaculture is that all-male populations are desirable because males demonstrate superior growth characteristics compared to females. In addition, culture of monosex populations prevents reproduction and results in a uniform fish size, given that females reach sexual maturity earlier. With the intention of solving such situation, the synthetic steroid 17α-methyltestosterone (MT), a derivative of testosterone is frequently used to masculinize tilapia juveniles in order to later be used in commercial aquaculture (Owusu-frimpong and Nijjhar, 1981; Green *et al.*, 1997; Abucay and Mair, 1997; Gale *et al.*, 1999).

Many strategies were performed to immprove tilapia production intensity without using hormone, such as the use of super male tilapia (Mair *et al.*, 1997), Periodic harvesting of tilapia fry and fingerlings Mair, (2002), Manual separation of male from female fish (Hickling, 1963), ….etc. Although, the use of MT treatment to produce monosex tilapia was the popular and successful practice all over the world (Shelton *et al.*, 1999).

2.1. Fish growth and hormone regulations:

Physiologically, growth is usually defined as an increase in cell number and/or cell size concomitant with a positive change in energetic (caloric) content of the organism (Johnston, 2001). He also, stated the biotic and abiotic factor controlled fish growth, these factors range from temperature, photoperiod, oxygen concentration, salinity, medium density, water quality, and water flow, through food quality, availability, animal density, reproductive phases and pollutants. Further, inter- and intraspecific competition, social interactions, predation, and disease can impact growth rates. Finally, genetic factors may be involved at every step in this process and always are an overriding consideration when discussing factors affecting growth.

In addition, fish growth doesn't follow the same pattern for other vertebrate, for two reasons. First, fish growth continues throughout their lives (absolute growth rate). Although the relative growth rates are decreased with age (Dutta, 1994). Second, fish muscle grows by hyperplasia as well as by hypertrophy. The post-hatch hyperplasia is a feature that sets the fishes apart from all other vertebrates, where hyperplastic muscle growth occurs only in very early ontogenetic stages and muscle fiber number is supposed to be fixed at birth-subsequent muscle growth is therefore primarily due to hypertrophy of existing muscle fibers (Alfei *et al.*, 1994; Johnston, 2001).

Many intrinsic factors regulate muscle growth, some of it, non-hormonal such as location of muscle in the fish body (Van-Leeuwen, 1995), Exercise which direct nutrients to build muscle (Johnsson *et al.*, 1996) and hormonal factors. A number of endocrine and paracrine factors affect muscle growth in fishes, and some of these principles are listed in (Table 1) according to (Johnston, 2001).

Table (1): Effects of hormones on muscle growth in fishes[1].

Hormone	Effects
Growth hormone	Increase protein synthesis; increase amino acid uptake and incorporation; increase lipolysis; affects reproduction.
Growth hormone transgenics	Early maturation
IGF-1	Hypoglycemia; increase protein synthesis; decrease protein degradation; increase amino acid uptake; increase cell proliferation.
Insulin	Increase amino acid uptake; increase protein synthesis; antilipolytic
Glucagon	Increase amino acid uptake
Prolactin	Increase intestinal amino acid transportarion; increase protein synthesis
Thyroid hormone	Metamorphosis; increase amino acid uptake; increase food conversion efficiency; positive nitrogen balance; increase protein synthesis.
Steroids **Estrogen** **Androgen** **Corticosteroids**	 Decrease myosin synthesis Increase growth performance; increase protein synthesis; increase intestinal amino acid uptake Increase plasma amino acids; increase proteolysis
Anti-SRIF	Blocks effect of growth hormone
B-Adrenergic agonists	Increase Protein synthesis; increase plasma fatty acids; increase muscle lipids

[1]Adapted from Johnston (2001); IGF-1: Insulin like growth factor one; Anti-SRIF: Anti-Somatostatin.

2.1.2. Steroid hormone:

Testosterone is the most important androgen secreted by the testis. The major source (95%) being the interstitial cells of Leydig (Howell and Shalet, 2001). The adrenals contribute to the rest (5%) of testosterone, in male 97–99% of total testosterone is bound to sex hormone binding globulin, and approximately 1-3% remains free and readily available for physiological needs.

Dihydrotestosterone is the other potent androgen secreted by the testes via converting testosterone in many target tissues to the much active dihydrotestosterone by the enzyme 5 α-reductase. The masculinization of the fetus occurs under the influence of dihydrotestosterone (Hinman, 1993). Moreover, testosterone is the biosynthetic precursor to both androgens and estrogens in teleosts (Baroiller *et al.*, 1999). The aromatase enzyme is the responsible for production of estrogen. Where, this enzyme is a cytochrome P-450 hemoprotein that catalyzes the conversion of androgens to estrogens (Eng *et al.*, 2001).

The testes in addition to producing the above mentioned androgens also produce androstenedione and dehydroepiandrosterone that are considered to be weak androgens. Dehydroepiandrosterone, regarded as the 'fountain of youth', was isolated in 1934 and is the major secretory product of adrenal gland, although the testes produce a small quantity. After production and secretion from these glands, the potentiality of this hormone to enter the androgenic pathway depends on the individual's medical condition, age and sex, for every individual has a unique biochemical composition. Dehydroepiandrosterone is metabolized to form dehydroepiandrosterone Sulphate, and both hormones are metabolically interconvertible by the action of the enzymes sulphotransferase for conjugation and sulphatase for hydrolysis, present in many tissues (Baulieu, 1996).

Testosterone is much more than a sex hormone (Seal, 2009). Anabolic steroids, especially androgens, are powerful growth promoters in fishes, especially in juveniles (Zohar, 1989). There are testosterone receptor sites in cells throughout the body, most notably in the brain and heart. Youthful protein synthesis for maintaining muscle mass and bone formation requires testosterone. Testosterone improves oxygen uptake throughout the body, helps control blood sugar, regulate cholesterol and maintain immune surveillance. The body requires testosterone to maintain youthful cardiac output and neurological function.

Furthermore, Gauthaman *et al.* (2002) they reported that androgens have a major role in the growth and differentiation of many tissues in addition to the organs of reproduction, testosterone is the main hormone having nitrogen-retaining (anabolic) properties which increases lean body mass and body weight. The administration of exogenous steroids can be effective in controlling sexual development (Al-ablani and Phelps 2002).

2.2. Mono sex tilapia:

The sex determination mechanism is driven by specific genes that control only the "initial decision" of phenotypic gonad gender, but this initial instruction that directs the gonad sex differentiation process can be overruled later on, by numerous intrinsic and extrinsic factors (Hayes, 1998). The fact is that at the moment of hatching in many species of fish, the gonads are not fully differentiated, and in other species are still in the process of tissue arrangement in the body cavity (Strussmann and Ito, 2005). In gonochoristic species such as tilapia, two major hypothesis of early gonadal development are suggested. In one case the undifferentiated gonad develops directly to ovary or testis, as seen in tilapia. Alternatively all individuals initially develop ovarian tissue, but later gonad masculinization can occur in some individuals. In the second case the gonads are initially intersex prior to differentiation into either testis or ovaries (Devlin and Nagahama, 2002). During the specific critical periods of early gonad development, changes in sex hormone levels can affect the final sex independently of the genetic sex (Andersen *et al.*, 2003). Since the process can take place at different stages in the life span of the individual species (for instance at prehatching in Japanese medaka or at the 2-year in sturgeon), there is a time window where external manipulation of several variables can re-direct the gonad differentiation process. In general, the most sensitive period is just prior to or concomitant with the initial histological differentiation of the primary gonad (Devlin and Nagahama 2002).

One of the main factors involved in directing the gonad differentiation process are sex steroids hormones (Piferrer, 2001). Such information is inferred based on multiple experiments in which administration of exogenous androgens or estrogens has successfully skewed the phenotypic sex ratio to the desired sex (Baroiller and D'cotta 2001; Strussmann and Nakamura 2002). The role of androgens in sexuality is unequivocal. Embryonic differentiation of the fetus into a male and its subsequent growth along this line is essentially due to the presence of physiological amounts of androgens (especially testosterone and its metabolite dihydrotestosterone) in the body (Baskin *et al.*, 1997).

The metabolism of steroids hormone during steroid sensitivity period in sex differentiation process of *O. niloticus* larvae was studied by (Rowell *et al.*, 2002). The authors found that testosterone synthesis increased in the XY genotype from day 8 to 13 days post fertlization. Meanwhile, in the XX genotype testosterone didn't increased, and the YY genotype showed intermediate testosterone synthesis to that of the XY and XX genotypes. Estrogens were not synthesized by any genotype.

Owusu-frimpong and Nijjhar (1981) reported that all male tilapia fry can be obtained via feeding undifferentiated *O. niloticus* fry with MT a dose of 50 mg/kg diet for four weeks. The efficacy of the hormone of choice MT depends on various factors, such as dose, timing and duration of treatment, and mode of administration (Mirza and Shelton, 1988).

Wahbi and Shalaby (2010) studied the effect of over dose of MT 60, 120 and 200 mg MT/kg feed for 21 days. The results showed that the highest male percent 89.2% were recorded with 60 mg MT/kg feed compared with 45.9% of control group. Meanwhile, the highest doses 120 and 200 mg MT/kg feed increased the neutral gonads and inhibition of oogenesis in dose dependent manner, also the histological investigation of mater reversed male after six months showed dominant spermatogengic cyst in 60 mg MT treatment compared to predomination of spermatozoa in control.

2.2.1. Advantages and disadvantages of mono sex tilapia:

Production of a single sex population is advantageous as it overcomes the problem of overpopulation and stunting as found in a bi-sex culture environment. In addition, male Nile tilapia has about twice the growth rate and a larger body size as compared to female tilapia (Green *et al.*, 1997).

It is of prime importance in order to report the MT residues in the treated tilapia at marked size. Goudie *et al.,* (1986) reported that tilapia fry fed for 30 days a feed containing radioactive labeled MT showed a rapid depletion of radioactive MT from tilapia muscle with only a trace of MT could be found. Moreover, Rizkalla *et al.* (2004) found that testosterone concentrations in muscle samples taken from the monosex fish did not differ from the untreated controls and the concentrations were below the detectable level (3ng/g).

Finally, the introduced of monosex tilapia as alien species for aquaculture in the new environment was considered a solution for the escapees fish to the ecosystems and the risk of genetic impact with native populations of tilapias, were the MT treated fish have much lower potential to reproduce compared to normal fish including interbreeding with wild tilapia stocks (Mair *et al.*, 2007).

The disadvantages of monosex tilapia waylay in the probability of human health hazard of MT and the environmental impact. El-Neklawey *et al.,* (2009) examined the MT residues in flesh of farmed fish of Tilapia (*O. niloticus*) and Silver carp (*Hypophthalmichthys molitrix*) using Thin Layer Chromatography, as well as it was quantitatively assayed by Radio-Immuno-Assay. The authors detected MT residues in 24% and 4 % of tilapia and carp sample; respectively. The hormonal residues of testosterone was ranged from 3.25 to 34.9 ng/g with mean value 4.22±1.1 ng/g in positive samples of Tilapia, while carp sample showed 22.0 ng/g of MT.

The MT is a suspect human carcinogen and is known to induce nonmalignant tumors in the liver (Soe *et al.*, 1992). The chronic exposure of humans to MT can cause adverse health effects such as hepatotoxicity; therefore, ingestion of MT residue in treated fish may be a potential hazard to human consumers. The quantity of MT residue in fish tissue will depend on its dosing history and its pharmacokinetics characteristics (Vick and Hayton 2001).

There is growing attention being given to the impact of pharmaceutically active compounds, including hormones, released into the environment via waste water discharge (Heberer, 2002). Use of the standard MT treatment for all-male tilapia production will result in the release of this androgen into the water system for up to one month. The hormone will be released from uneaten feed, faecal and urinary excretion (Johnstone *et al.*, 1983). Since it is very common to overfeed the fry, residual MT in the MT-impregnated food from the masculinizing process may accumulate in the ponds and be released into the receiving water body when the pond water is released or when the ponds are cleaned (Homklin *et al.*, 2011).

The few studies available in the literature shed some light on the occurrence of MT in the sediments and pond water. Fitzpatrick and Contreras-Sánchez (2000) reported an MT aqueous concentration of 3.6 µg L^{-1} in a fish masculinization pond at the end of the masculinization process which eventually decreased to background level (between non detectable and 0.02 lg L^{-1}) in a week. Meanwhile, MT in the sediment of the pond persisted at concentrations between 2.8 and 2.9 ng g^{-1} for nearly 3 months after the end of the masculinization process.

Even if water from a masculinization pond treated with a biological filter, the MT residual was high enough to achieve 81–100% sex inversion of common carp (*Cyprinus carpio* L.) progeny (Hulak *et al.*, 2008).

The mean environmental impacts of MT were endocrine disrupting effect for wild species. Indeed, endocrine modulating substances are of high ecotoxicological relevance, due to their potential adverse influence on the reproduction of organisms and consequently, on the existence of whole populations (Zerulla *et al.*, 2002). Korsgaard, (2006) found that vitellogenin in female eelpout (*Zoarces viviparous*) that expresses the female characteristic was found to decrease when exposed to 10–500 ng L^{-1} of MT for 10 days. Other studies demonstrating the endocrine disrupting characteristics of MT include a decrease in the fecundity and fertility of medaka fish when exposed to about 46 ng L^{-1} of MT (Kang *et al.*, 2008). In a case of poultry, a decrease in the egg-laying rate of female Japanese quails (*Coturnix cotumix japonica*) and the fertility of male Japanese quails when exposed to 50–110 mg L^{-1} of MT for 3 weeks (Selzsam *et al.*, 2005).

2.3. Phytochemicals:

Phytochemicals contained in herbs may enhance the innate immune system, possess antimicrobial capabilities, and are redox active molecules with antioxidant characteristics that may help to improve the general physiological condition of fish (Chakraborty *et al.*, 2013). The phytochemicals can also modulate endocrine function and change the sex ratio of the exposed Zebra fish fry (Nakari and Erkomaa 2003).

Additional reported biological activities for phytochemicals compounds are the ability to interact with protein phosphorylation, iron chelation, and a series of enzymatic reactions (Bonina *et al.*, 1996; Boyle *et al.*, 2000). The most relevant biological property of phytochemicals is their ability to act as antioxidants. The oxidative stress in general, is conferred by a high number of hydroxyl substitutions which has a direct effect on the donating ability of hydrogen (Pietta, 2000; Kim, 2001).

In addition, flavonoids may regenerate other antioxidants such as tocopherol by donating a hydrogen atom to the tocopheryl radical (Boyle *et al.*, 2000). In the public health domain, consumption of phytochemicals is associated with a decreased risk of cardiovascular disease by protecting against oxidative cell damage (Reiterer *et al.*, 2004). Such action is facilitated by the stable structure and low molecular weights of phytochemicals that can pass through cell membranes (Ososki and Kennelly, 2003).

2.3.1. Ginseng (*Panax ginseng*):

Ginseng is a perennial plant indigenous to both Asia and North America (Jia and Zhao, 2009). It is taxonomically organized with the family Panax which consists of 13 species of ginseng that have been identified to date (Choi and Wen, 2000).

Morphologically, the ginseng plant is composed of a root, short upright rhizomes, stems, leaves, berries and buds, which blossom into flowers after three years (Wen, 2001; Choi, 2008). It is a self- pollinating plant whose roots grow underground year-round, while the aerial growth terminates in the fall and resumes in the spring (Choi, 2008). Ginseng is normally harvested between three and six years of age, which is considered a measure of product quality in the Asian market (Yap *et al.*, 2005; Jia and Zhao, 2009).

2.3.1.1. Chemical Composition:

The ginseng plant contains several hundred components, making it difficult to identify a single agent responsible for its efficacy (Hui *et al.*, 2009). Active components of ginseng is the triterpenoid saponins referred to as ginsenosides, the polysaccharides and the polyacetylenes (Christensen, 2009). also, the ginsenosides are considered the most pharmacologically active and are consequently the main focus of ginseng research (Jia and Zhao, 2009; Luo and Luo, 2009). Its chemical constituents include peptides, fatty acids, vitamins and minerals (Luo and Luo, 2009).

The metabolic process occured via intestinal microbiota alter the chemical profile and corresponding bioactivities of herbal medicines. Where, most herbal medicines are orally administered, intestinal microbiota may play an important role in mediating the metabolism bioactivity of herbal medicines (Wang *et al.*, 2011). For instance, the microbiota-mediated metabolism of ginsenosides in ginseng and red ginseng and the majour metabolite compound K and its pharmacological advances are anticancer, antidiabetic and anti-inflammatory effects.

2.3.1.2. Effect on growth performance:

Goda, (2008) studied the effect of ginseng extract supplementation (50, 100, 150, 200, and 250 mg/kg diet) on growth performance, feed utilization, and hematological indices of Nile tilapia, *O. niloticus*. The results indicated that growth performance and feed utilization efficiency of Nile tilapia were significantly higher in all treatments receiving ginseng supplemented diets than the control diet.

In the contrast, Choi *et al.*, (2010) examined the effect of dietary ginseng by product alone or with mixture of garlic extract, yeast and filler on the growth of juvenile olive flounder (*Paralichthys olivaceus*). The all studied concentration (0.5, 1 and 2%) of the two supplementations reduced the growth and feed utilization of treated fish.

Yan *et al.*, (2011[a]) found that the use of 0.1% wild-ginseng adventitious root added to the diet could increase growth performance, and induced a linear decrease in abdominal fat and in broilers chicken. Furthermore, in laying hens wild-ginseng adventitious root meal increased egg production, also, reduces saturated fatty acids and increases the poly unsaturated fatty acids in egg yolk (Yan *et al.*, 2011[b]).

Ao *et al.*, (2011) evaluated the effects of fermented red ginseng (1, 2 and 4 g/kg diet) on growth performance, apparent nutrient digestibility, blood hematology and meat quality in finishing pigs. Throughout the whole period of the trial, there were no effects of ginseng addition on average daily gain or gain to feed ratio. Pigs fed 2 g/kg diet had lower average daily feed intake than those fed control diet during 0-4 weeks while 2 g/kg diet and 3 g/kg diet treatments decreased average daily feed intake compared with control treatment both during 5-8 weeks and the entire experiment. No differences were observed in apparent nutrient digestibility and blood hematology, while partially improved meat quality in finishing pigs. Furthermore, Ginseng may improve flesh quality where it shown to be regulates the lipid metabolism and level in the animal body.

2.3.1.3. Effect on endocrine systems and sex status:

Tsai *et al.*, (2003) reported that ginsenoside-Rb1 increases luteinizing hormone secretion by acting directly on rat anterior pituitary gland cells. *P. ginseng* and *Eleutherococcus senticosus* (*Siberian ginseng*) have a long history of traditional use and were commonly prescribed to enhance male virility and fertility (Sinclair, 2011).

Moreover, Yang *et al.*, (2011) investigated the effect of *P. ginseng* on spermatogenesis and the regulation of glial cell derived neurotrophic factor, which has a crucial role in spermatogonial stem cell maintenance. *P. ginseng* was administered to 8 week old male Wistar rats (1.0 g/kg/day) for 56 consecutive days, the sperm formation period of the rat. The *P. ginseng* treated group had significantly enhanced sperm counts, glial cell derived neurotrophic factor mRNA level and protein level.

2.3.1.4. Effect on oxidative stress and antioxidants:

Voces *et al.,* (2004) reported that the administration of ginseng extract was able to protect muscle from exercise-induced oxidative stress, respective of maintain glutathione status and reduced lipid peroxidation in rats. In the *in vitro* study, Kim *et al.,* (2007) reported that ginseng protects cell viability against H_2O_2-induced oxidative damage, and enhanced the activities of Super oxide dismutase (SOD) and catalase (CAT) in dose dependent manner.

The administration of ginseng extract (100 mg/kg BW) resulted in a significant improvement of fertility parameters and testicular antioxidants together with a decrease in malondialdehyde (MDA) and testicular pathological signs including degenerative changes of the seminiferous tubules in rendered diabitic rats (Sawiress *et al.,* 2011).

El-Khayat *et al.,* (2011) studied the antioxidant effect of *P. ginseng* on diabetic rate. The authors found that liver antioxidants were decreased in the diabetic group while they were significantly increased after treatment with ginseng.

Kim *et al.,* (2011[a]) examined the protective effects of korean red ginseng (250 mg/kg) against hepatotoxicity induced by Aflatoxin B1 using SOD, CAT, glutathione peroxidase (GPx), and MDA determination. SOD, CAT, and GPx activities were high as compared to the Aflatoxin B1 alone group. These results indicate that ginseng may have protective effects against hepatotoxicity induced by Aflatoxin B1 that involve the antioxidant properties of ginseng.

Kim *et al.,* (2011[b]) investigated the antioxidant effects of *P. ginseng* (1 and 2gm/day) on healthy human volunteers for 4 weeks. The authors found that 2g of *P. ginseng* improved total glutathione content and GSH activity. However, no significant alterations were observed in total oxidative stress, CAT, SOD, and GPx activities.

Ramesh *et al.,* (2012) found that administration of the fermented *P. ginseng* extract to aged rats resulted in increased activities of SOD, CAT, GPx, GSH and glutathione *S*-transferase (GST) as well as elevation in glutathione, ascorbic acid and α-tocopherol levels.

2.3.1.4. Effect on blood hematology and biochemical:

Goda, (2008) studied the effect of ginseng extract supplementation on hematological indices of Nile tilapia. The results showed that red blood cells counts, hematocrit, and hemoglobin significantly increased with increasing dietary ginseng levels compared to those of the control diet. The same trend was observed for total plasma protein and total plasma globulin levels.

Choi *et al.,* (2010) examined the effect of dietary ginseng by product alone or with mixture of garlic extract, yeast serum chemistry and lysozyme activity. The all

studied concentration (0.5, 1 and 2%) of the two supplementations improves serum glucose, triglyceriede, glutamate pyruvate transaminase. Also, lysozyme activity was higher in fish feed ginseng by product (0.5 and 1%) than control and other treatments. Wild-ginseng adventitious root decreased serum cholesterol in broilers chicken and laying hens (Yan *et al.*, 2011[a&b]).

Kim *et al.*, (2011[a]) examined the protective effects of korean red ginseng (250 mg/kg) against hepatotoxicity induced by Aflatoxin B1 using liver-specific serum marker analysis. In the ginseng pre-treatment group, serum alanine aminotransferase, aspartate aminotransferase, and MDA levels were low as compared to the Aflatoxin B1 alone group.

Song *et al.*, (2012) studied lipid metabolic effect of Korean red ginseng extract in mice fed on a high-fat diet, and found that ginseng reduces the levels of cholesterol, LDL, serum triglycerides, and atherogenic indices. Also, Levels of leptin, adiponectin and insulin, which regulate glucose and lipid metabolism, were impaired profoundly by high-fat diet. However, ginseng treatment brought these levels back to normal. Also, Ramesh *et al.*, (2012) reported that aspartate aminotransferase, alanine aminotransferase, urea and creatinine were reduced with administration of fermented *P. ginseng* extract to aged rats.

2.3.2. Tribulus (*Tribulus terrestris*):

Tribulus (*Tribulus terrestris*) is a plant belonging to Zygophyllaceae family, also called gokshura, puncture vine, small caltrops, Chih-hsing in China and goathead in USA (Tutin *et al.*, 1968), meanwhile, in Arab countries called Al-Gutub, Qutiba, Hasak or Ders El-Agouz (Al-Ali *et al.*, 2003), or Al-Hassage or Al-Kutub (Hussain *et al.*, 2009).

Morphologically, the Tribulus is a 10-60 cm high, annual herb with pinnate leaves and yellow flowers, its carpel fruits are stellate shape (Tutin *et al.*, 1968). This plant is widely distributed in Turkey, China, Japan, Korea, the western part of Asia, the southern part of Europe and Africa (Sahin and Duru 2010). In Egypt, Temraz *et al.*, (2006) and El-Tantawy *et al.*, (2007) collected, Tribulus *alatus*, from Al Azhar University garden, Nasr-city, Cairo, Egypt and identified it by department of botany, faculty of science, Cairo University. Also, Hammoda *et al.*, (2013) collected Tribulus from train railway and along the Mahmudiya Canal, Alexandria, Egypt. The plant material was identified by Faculty of Science, Alexandria University, Egypt.

2.3.2.1. Chemical composition:

Tribulus contains biologically active substances as steroids, saponins, flavonoids, alkaloids, unsaturated fatty acids, vitamins, tannins, etc. (Adaikan *et al.*, 2000). Tribulus contains a number of different substances known as steroidal saponins.

Protodioscin is the most dominant saponin in Tribulus (Ganzera *et al.*, 2001). Also, Kostova and Dinchev (2005) reported that the main active components of this plant are saponins of the furostanol type, termed protodioscin.

Tribulus extract, which contains a non-hormonal Phyto-dehydroepiandrosterone, is the complementary drug of choice to cure sexual dysfunction in men (Adimoelja, 2000; Adimoelja *et al.*, 2005). In Egyptian, Temraz *et al.*, (2006) studied the phytochemicals of *T. alatus* and isolated six steroidal glycosides from its aerial parts, together with one known cholestane, one spirostane, and six flavonol glycosides.

2.3.2.2. Effect on growth performance:

The effects of the Tribulus extract on body weight have been studied by Turan and Çek (2007) who studied the effects of different concentrations of Tribulus extract (0, 0.1, 0.2, or 0.3 g/L water for 30 days) on growth and survival of African catfish, *Clarias gariepinus.* The best growth was obtained in the 0.3 g Tribulus treatment. The Tribulus improved survival rate from 72% in the 3g Tribulus group to 80% in the 9g Tribulus during treatment and didn't significantly differ from the control.

New-born guppies (*Poecilia reticulata*) treated with Tribulus extract exhibit successful growth acceleration comparing to control group especially with the concentration 0.1 and .15 g/L which reached to 4.5 time more than control for the two concentration (Çek *et al.*, 2007[a]). Moreover, Çek *et al.*, (2007[b]) investigate the effect of higher doses of Tribulus extract (0.0, 0.10, 0.20 and 0.30 g/l) on growth and survival of convicted cichlid, the 0.3 g/l Tribulus extract improved growth rate 1.6 times more than control, the survival rate was uniformly high in control and treated group ranging from 88.57 to 90%.

Some studies about anabolic Tribulus affects performed on chicken, such as Duru, (2005) who used Tribulus extract (60 and 120 ppm) as feed supplement on broiler chicks for 21 days. Tribulus extract didn't affect growth performance and body parts. Moreover, Sahin and Duru, (2010) investigate the efficacy of Tribulus extract in higher doses (180 and 360 ppm in basal diets) on growth and muscle building in broiler chicks. Also, their findings showed that Tribulus extract didn't affect growth performance and muscle building but decreased ileum, jejunum and empty gut weights.

Gültepe *et al.*, (2014) studied the effect of Tribulus extract at levels of 0, 200, 400 and 600 mg/kg diet on Nile tilapia. The results showed that growth performance and feed utilization of Nile tilapia were significantly higher in all treatments fed with Tribulus extract supplemented diets than in the control diet. There were no significant differences in body composition of fish.

2.3.2.3. Effect on endocrine systems and sex status:

Tribulus widely used to raise testosterone levels safely and naturally and is rumored to be the secret behind the success of many top Bulgarian weightlifters (Bucci, 2000).

The effect of Tribulus extract was investigated on sex reversal on ovoviviparous guppy fish, *Poecilia reticulate*. The authors treated newly born offspring once weekly by immersion for two month with Tribulus (0.0, 0.05, 0.1 and 0.15 g/L) and found that the significant effective dose of Tribulus in sex reversal was 0.15 g/L which ensured maximum male ratio (80%) compared to control 50%, the other doses increased male ratio 58.25 and 59.77% with 0.05 and 0.1 g/L, respectively (Çek *et al.*, 2007[a]).

Also, the same authors (Çek *et al.*, 2007[b]) conducted a sex reversal study on newly hatched convict cichlid, *Cichlasoma nigrofasciatum*, using higher doses (0.0, 0.10, 0.20 and 0.30 g/l) of Tribulus extract by immersion once weekly for 2 months. The results showed that 0.30 g/l Tribulus was the most effective in terms of masculinization, resulting in a maximum male ratio of 87.23%, also 0.10 and 0.20 g/l Tribulus produced significant male ratio 79 and 85% compared to control, respectively. Moreover, tests histology proved that Tribulus extract stimulated spermatogenesis in convict cichlid.

Turan and Çek (2007) studied the effects of different concentrations of Tribulus (0, 0.1, 0.2, or 0.3 g/L water for 30 days) as a masculinization agent on African catfish, *Clarias gariepinus*. The male percent increased from 55.56% in control to 74.54, 79.22 and 80.42% with Tribulus treatments, respectively. Moreover, morphological and histological examinations of the gonads in all groups revealed no intersex fish and no damage to the testes or ovaries were observed.

The Tribulus effects on sexual performance not only confined on mature animal but also found to stimulate function of the tests in immature albino rats, where Bashir *et al.*, (2009) found that 70 mg/kg body weight daily for 20 days increased paired testes weight, seminiferous diameter, early spermatids in seminiferous tubules. Moreover, spermatogenesis was present at an advanced stage in the experimental group as compared to the control.

Kavitha and Subramanian (2011[a]) assessed the influence of Tribulus (0, 100, 150, 200, 250, and 300 mg/l) on the activities of testicular enzyme in, *Poecilia latipinna*, fish in lieu of reproductive manipulation. Tribulus induced the testicular enzyme activity such as Sorbitol dehydrogenase, lactate dehydrogenase activities, Acid phosphatase, alkaline phosphatase activities and Glucose-6-phosphate dehydrogenase activity, the activity of these enzymes was markedly higher in 200 and 300 mg than those of control, that may aid in the male reproductive functions. The same previous researchers (Kavitha and Subramanian, 2011[b]) evaluate the efficacy of these

concentrations on masculinization of *Poecilia latipinna* and found that a dose-dependent masculinization occurs on administration of Tribulus, which improved male ratio. These results harmonize with the increase of testosterone and luteinizing hormone (LH) hormone in the treated fish.

2.3.2.4. Effect on oxidative stress and antioxidants:

Tribulus fruit extract scavenged reactive oxygen species induced by γ-radiation in a concentration-dependent manner, also showed protection against oxidative stress-induced apoptosis (Pandey *et al.*, 2007).

Kadry *et al.*, (2010) investigated the antioxidant activity of alcoholic extract of *T. alatus*, via determination of blood glutathione, serum ascorbic acid and serum SOD in rats. Their results showed that all groups of rats treated with aerial parts without fruit, fruits and total herb showed a significant increase in all measured parameters.

In 2011, Kavitha *et al.*, studied the hepatoprotective activity of Tribulus (250 mg/kg) extract against acetaminophen-induced toxicity (500 mg/kg) in *Oreochromis mossambicus*. The acetaminophen induced a significant rise in the tissue-damaging level, and the antioxidant level was discernible from the enzyme activity modulations such as SOD, CAT, GPx, GSH, GST, lipid peroxidase and reduced glutathione (GSH). The elevated levels of these enzymes were significantly controlled by the treatment of Tribulus extract.

Recently, Hammoda *et al.*, (2013) investigated the phytochemical and antioxidant properties of Tribulus. The authors isolated two new oligosaccharides and a new stereoisomer of di-p-coumaroylquinic acid along with five known compounds. The antioxidant activity represented as 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity revealing that the di-*p*-coumaroylquinic acid derivatives possess potent antioxidant activity and so could be the major constituents contributing to the antioxidant effect of the plant.

2.3.2.4. Effect on blood hematology and biochemical:

Jagadeesan *et al.*, (2005) reported that methanol fractions of Tribulus fruit extract caused slowly recover the marked fall in the level of bio-chemical constituent in the liver tissue due to mercury toxicity in mice. Kavitha *et al.*, (2011) studied the hepatoprotective activity of Tribulus extract against acetaminophen-induced toxicity *O. mossambicus*. The plant extract showed a remarkable hepatoprotective activity against acetaminophen-induced hepatotoxicity where maintain the normal level of the enzyme activity modulations such as glutamate oxaloacetic transaminase, glutamate pyruvic transaminase, alkaline phosphatase, acid phosphatase, glucose-6-phosphate dehydrogenase, lactate dehydrogenase.

Gültepe *et al.,* (2014) studied the effect of Tribulus extract at levels of 0, 200, 400 and 600 mg/kg diet on Nile tilapia. The results showed that, hematocrit, hemoglobin, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and myeloperoxidase variables were not significantly affected by herb extract. However, mean corpuscular volume (MCV) value and lysozyme activity increased with increasing Tribulus extract. Serum albumin, total protein and triglyceride levels were not affected by Tribulus extract. Serum globulin and glucose levels increased and cholesterol levels decreased in Nile tilapia fed with herbal supplemented diets.

2.3.3. Date palm pollen:

Date palm (*Phoenix dactylifera* L.) belonging to family *Arecaceae*, called Nakhla and the tree of life by the Arab countries, also, considered as one of the oldest cultivated fruit trees (Vyawahare *et al.,* 2009). Date palm is native to the Middle East region over centuries ago (Copley *et al.*, 2001). In Folkloric practice, date represents an essential meal in some Arab area (Miller *et al.*, 2003).

Pollens are the male reproductive cells of flowers (Hassan, 2011). The early Egyptians and ancient Chinese used pollen as a rejuvenating medicinal agent. It has been called a "fountain of youth" (Kroyer and Hegedus, 2001). The beneficial health and nutrition values of *P. dactylifera L.* for human and animal consumption have been claimed for centuries (Barreveld, 2010).

The experimental animals treated with date palm pollen (120 mg/kg daily) had normal food and water intake and were active without any sign of ill health; there was no mortality seen, indicating that date palm pollen administration didn't have any negative effect on the survival of experimental animals (Iftikhar *et al.,* 2011).

2.3.3.1. Chemical composition:

Phytochemical screening of dried date palm pollen grains indicated the presence of sterols (mainly brassionsteroids), triterpenes, saponins, proteins, carbohydrate and/or glycosides and lacked volatile substances (Mahran *et al.,* 1976). Moreover, the analysis of date palm pollen showed the presence of estrone, α-amirin, triterpenoidal saponins, flavonoids and a crude gonadotrophic substance (Mahran *et al.,* 1985; Bennet *et al.,* 1996).

Abbas and Ateya (2011) analyzed the Egyptian date palm pollen using column chromatography and revealed the presence of estrone, estradiol and estriol from the hexane fraction. Also, besides rutin, additional four flavonoids from the ethyl acetate extract were isolated.

Furthermore, the date palm pollen active components, Hassan (2011) determined the nutritive value of Egyptian date palm pollen grains. The obtained results

indicate that the proximate chemical composition of date palm pollen grains were 28.8% moisture, 31.11% crude protein, 20,74% fat, 13.41% carbohydrate, 4.57% ash, 1.37% fiber. Moreover, the chemical composition revealed the presence of logical amount of vitamin A, E and C. It also represents a good source of minerals such as Boron, Zinc, Selenium, Iron, Molybdenum, copper, Manganese, Cobalt and Nickel. The major essential amino acids were leucine and lysine and the predominant fatty acids were palmitic, linoleic and myristic.

2.3.3.2. Effect on growth performance:

Wang *et al.,* (2007) investigate the trophic effect of bee pollen (1.5% for 6 weeks) on growth and development of small intestine in broiler chickens. Their investigation showed that bee pollen group grew significantly faster than those in the control group, by 35.1%. The histology of small intestine showed that villi from the duodenum, jejunum, and ileum were longer and thicker in the pollen group. Furthermore, the small intestinal glands were developed at a higher density in the pollen group, and the depth of the glands was significantly increased by bee pollen.

Attia *et al.,* (2011[a]) reported that rabbit offspring received a water solution containing 0, 100, 200 and 300 mg bee pollen/kg body weight, twice per week for 12 week, showed improvement in growth performance in bee pollen group than control. The Bee pollen at 200 mg/kg BW reveal increase in weight gain and survival rate and reduced feed intake and feed conversion ratio of offspring.

Attia *et al.,* (2011[b]) found that bee pollen at 200 mg/kg body weight of rabbits significantly increased body weight of does, conception rate, milk yield and litter size; and helps outstanding of does when dosed before 1 week of mating and till 1 week after mating. Moreover, the treatment with bee pollen significantly increased kit growth and their survival rate until weaning.

Abbass *et al.,* (2012) investigate the supplementation of Nile tilapia, *O. niloticus,* diet with 2.5% of honeybee pollen for 21 days. The results showed that dietary honey bee pollen significantly improved length, specific growth rate, average daily gain and feed efficiency ratio. On the other hand, Hassan *et al.,* (2012) study the effect of 240 mg date palm pollen/kg body weight of adult male albino rats daily for 30 days, the weight of treated adult male rat didn't differ compared to control.

2.3.3.3. Effect on endocrine systems and sex status:

Marbeen *et al.,* (2005) studied the effect of 500 mg date palm pollen twice daily for 3 months on human male hormones. Their findings revealed that serum level of FSH, LH, and testosterone significantly increased compared to baseline data, the percentage of hormonal changes were (+77.962%), (+112.984%), and (+64.250 %) respectively.

An investigation by Bahmanpour *et al.,* (2006) showed that the aqueous suspensions of date palm pollen containing 30, 60, 120 and 240 mg/kg rat body weight, for 35 consecutive days, increase the blood level of testosterone and estradiol with all studied doses. The beak of testosterone and estradiol was achieved with 120 and 60 mg/kg, respectively. Moreover, consumption of date palm pollen suspensions improved the sperm count, motility, morphology, and DNA quality with a concomitant increase in the weights of testis and epididymis in dose dependent matter.

Iftikhar *et al.,* (2011) reported that date palm pollen in a single oral dose of 120 mg/kg daily for prepubertal albino rats didn't appear significant differences in testosterone levels after 18 days of treatment between control and experimental group, while after the elongation of treatment to 35 days, significant increase in serum testosterone levels was noticed compared to control group.

The adult rat treated orally with date palm pollen (240 mg/kg body weight, daily for 30 days) dramatically increased estradiol level, slightly increase testosterone level of normal rats, and improve sex organs weight, sperm count and motility. Co administration of date palm pollen with cadmium chloride significantly restored the reduction in sex organs weight and the decline in sperm counts and their motility as well as the decrease in testosterone level induced by cadmium chloride challenge (Hassan *et al.,* 2012).

Abbass *et al.,* (2012) reported that honeybee pollen (2.5% of feed) significantly increased testicular weight, gonadosomatic index and improved the semen quality of male Nile tilapia, *O. niloticus,* moreover, the testes histology showed accumulated sperms in seminiferous tubules. However, in female the effect of honeybee pollen was mild improvement in tested parameters. The authors concluded that keeping brood stock Nile tilapia on diet with 2.5% pollen before restocking into the breeding results in the highest rate of hatchability in female and fertilizing capacity in males.

2.3.3.4. Effect on oxidative stress and antioxidants:

Moreover, Abbas and Ateya (2011) the antioxidant activity of ethyl acetate fraction of date palm pollen via the DPPH radical method, and found that the extract possess a relatively strong antioxidant against DPPH radicals.

Treatment with date palm pollen (240 mg/kg body weight, daily for 30 days) ameliorate antioxidant status of rat via counteracted the increases in antioxidant systems in rat testis induced by cadmium chloride as assessed by restoration of GSH, SOD and CAT (Hassan *et al.,* 2012).

El-Neweshy *et al.,* (2013) found that rats treated with date palm pollen (40 mg kg^{-1}) orally, once daily for 56 consecutive days, restored the toxic effects of cadmium on the antioxidant systems (decreased MDA and increased GSH levels).

2.2.3.4. Effect on blood hematology and biochemical:

Al-Shagrawi, (1998) investigate the effect of date palm pollen grains (0.0%, 2.0% and 4.0%, for 35 days) on lipid of the plasma, liver and brain of rats, as well as fatty acid composition and the activities of liver function enzymes. The author found that significant reduction in plasma total cholesterol by 30.8% and 19.1%, total lipids by 39.1% and 39.86%, triglyceride by 6.9% and 41.8%, and low-density lipoprotein cholesterol by 54.7 and 21.8 in rats consumed modified diets containing 2.0% and 4.0% pollen grains of date palm, respectively. Moreover, the author found a significant elevation in plasma high-density lipoprotein - cholesterol of treated rats compared to the control. Lipid fractions of liver and brain in treated rats were also significantly lowered compared to the control. The liver function enzyme activities were significantly reduced in treated rats. Abbass *et al.,* (2012) concluded that honeybee pollen reduced Alanine aminotransferase in Nile tilapia *O. niloticus*, but aspartate aminotransferase, urea and creatinine didn't differ.