Dietary Acidifiers as Growth Promoters in Aquaculture

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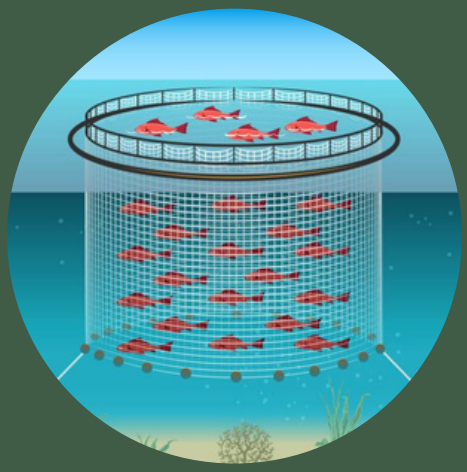

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1. INTRODUCTION

Aquaculture is an increasingly important option in animal protein production. Modern fish production has achieved phenomenal gains in the efficient and economical production of high quality and safety fish by the provision of nutritionally and economical effective diet for the different developmental stages of the fish species. Balanced diet is one of the major factors that world ensure success in the expansion and intensification of the aquaculture industry to increasing production of aquatic organism's culture (Storch *et al.*, 1984).

Public opinion and regulation authorities in most exporting countries now focus on the banned all antibiotic growth promoters (AGP) from aquaculture and livestock production (Lückstädt, 2006a). Therefore, aquaculturists in various parts of the world have continuously needed to develop diets for increased production of either the seed or the marketable size of economically important cultured fish species, by developing dietary alternative additives and strategies to replace antibiotic, such as: acidifiers, probiotics, prebiotics, enzymes, biogenic additives (Mccartney, 2005; Adams, 2005; Dibner, 2004; Dijk, 2004; Tovar-Ramírez *et al.*, 2010; Chiu and Liu, 2014).

In recent decades, acidifiers (especially organic acids) are routinely included in diets for monogastric animals in Europe in order to replace antibiotics as growth promoters (Papatsiros and Christodoulopoulos, 2011). Much of this interest arises from increased public awareness and objection against use of antibiotics as growth promoters in animal diets from 2006. Since that time appeared the importance of acidifiers and now Europe is the largest as well as the

fastest growing feed acidifiers market in the world (Lückstädt, 2007). Acidifiers consisting of organic acids and their salts, they improve the performance and the health of treated animals (Lückstädt, 2008). The nutritionists started to use organic acids for more than 30 years as suppress the growth of mould and bacteria and contribution to feed hygiene because they are growth promoters, antimicrobial activity, and reduce pH in the stomach and small intestine (Freitag, 2007). Nowadays the acidifiers are adding to food of human and many animals such as pigs specially in the weaning period (Ravindran and Kornegay, 1993; Lückstädt and Mellor, 2011), chicken (Smulikowska *et al.*, 2008) and fish like red sea bream *Pagrus major* (Hossain *et al.*, 2007). Organic acid salts as acidifiers in aquaculture is alternative efficient tool to achieve sustainable and economical fish and shrimp production due to their characteristics including: a) adjust the pH value in the gastrointestinal tract, increase the activities of digestive enzymes; b) improve the microflora in the digestive tract and feed palatability; c) inhibit the growth of harmful bacteria, enhance the immune function; d) increase animal performance, synergetic reaction with antibiotics; e) and reduce or prevent the occurrence of drug resistance (Lückstädt, 2006a). Some studies were performed using acidifiers in fish nutrition (Abdelhamid *et al.*, 1998; Bakr and Haggag, 2005; Zhou *et al.*, 2009) and shrimp nutrition (Tung *et al.*, 2006).

Tilapia fish are widely recognized as one of the most important fish species for freshwater aquaculture after carps and salmons (Zaghloul *et al.*, 2002). Several species of tilapia are cultured commercially, but Nile tilapia *Oreochromis niloticus* is the predominant cultured species worldwide, sometimes it called “the

aquatic chicken" because it is similar to chicken consumption. Nile tilapia fish is characterizing by high reproductive, grow rapidly on formulated feeds with lower protein levels, tolerate higher carbohydrate levels than many carnivorous farmed fish species, accept feeds with a higher percentage of plant proteins, relatively resistant to poor water quality, disease and hardy in nature, and given better growth both monoculture or polyculture (Abdelaal, 2000; Khattab *et al.*, 2001; Nandlal and Pickering, 2004). Egyptian production of Nile tilapia of nearly 705 490 tones in 2010 representing of 70 % of the total aquaculture harvest (1000000 tones) (GAFRD, 2010).

Fresh-water prawns are decapods crustaceans related to crabs and marine shrimp (Spotts, 1981). The giant freshwater prawn, *Macrobrachium rosenbergii* is native to Southeast Asia, south Pacific countries, northern Oceania, and western Pacific islands (New, 1982; 2002). *M. rosenbergii* has evolved to survive in the brackish water of the estuaries and the fresh-water Rivers, but in fact, it grows so much better in fresh water (Spotts, 1981). *M. rosenbergii* is desirable for a prawn culture, because has received considerable attention for its attractive characteristics as an aquaculture species such as ease of breeding in the captivity and hatching, high growth rates and absence of serious disease problems (Abdel Razek *et al*, 1998; Maclean and Brown, 1991).

Aquaculture nutritionists have given a growing attention to nutrition studies of *M. rosenbergii* that are necessary for the development of its culture (Habashy, 2003a). In nature freshwater prawn eat pieces of worms, snails, clam, fish, rice, wheat, beans, nuts, aquatic plants and some fruits (Ling and Merican, 1961) these can also

use in the aquarium. The aquaculture production of this species represented by 97.5% (195 000 tons) in 2002 from global production (200 000 tons), and it will exceed 400 000 tons by 2010. The largest production countries are China (99,111 tons), India (42,820 tons) and Thailand (30,000 tons) (FAO, 2006), and the most production was coming from monoculture (96%) while remaining farmers utilized polyculture with other prawns or some fish (Schwantes *et al.*, 2009; Asaduzzaman *et al.*, 2009). In Egypt, imported freshwater prawn from Thailand, and was initiated culture practice. In 1986, began culture trials at Maryut Fish Farming Company, near Alexandria, have been fairly successful (Abdel Razek, 1993; Sadek and El-Gayar, 1993). The success on the commercial production of *M. rosenbergii* post larvae (PL) depends on the efficient use of available food sources (Freeman, 1990). Lower efficiency of commercial prawn PL diets has been attributed to the limited knowledge of larvae nutritional needs (Sorgeloos and Léger, 1992; De Barros and Valenti, 2003). Currently, the cost of feed has emerged as a major factor influencing the profitability of the prawn farmers in Egypt. However, relevant practical diets information is need to be developing to stain higher growth, survival and feed efficiency of prawn in order to reduce costs and increase predictability of marketable size production.

Available results related with the use of organic acids or their salts for the improvement of growth, feed efficiency, digestibility and mineral utilization in aquaculture are limited and not clear. Some researchers have shown positive effects with dietary organic acids in improving growth rate and feed efficiency (De Wet, 2005a; Lückstädt, 2007), but others have found nothing or negative response (Gislason

et al., 1996; Ringø *et al*., 1994; Owen *et al*., 2006). The inconsistent results and highly variable responses may be due to several factors such as stage of growth, complexity of diet, source, and level of organic acids and fish health status.

There are only a limited number of published studies of the use of organic acids or their salts for growth promotion, dietary nutrient digestibility as well as feed efficiency in Nile tilapia and freshwater prawn. Therefore, this book was carried out the effect of dietary sodium lactate, calcium lactate and calcium propionate levels as organic acids salts on growth, nutrient digestibility, proximate body composition, feed utilization induce and histological changes of liver, pancreas and gonads of Nile tilapia, *O. niloticus* fingerlings and of hepatopancreas and gonads of giant freshwater prawn, *M. rosenbergii* PL.

The present Egyptian population is expected to grow from 82.0 million people in 2009 to 105 million by 2030 (Wijkstrőm, 2004). As a result, the demand for food including fish is increasing. The demand of aquatic products for human consumption will grow to 2.5 million metric tons (Mt) by 2030 (Wijkstrőm, 2004) from its present production level of 1.4 million metric tons (GAFARD, 2010). This goes beyond total capture fisheries supply (400,000 metric tons in 2010). The shortfall in supply will largely filled in through aquaculture.

Tilapia is the most representative variety of freshwater aquaculture crops and one of the most widely cultured species in Egypt. The total aquaculture production of tilapia increased from

24,916 Mt in 1990 to 705,000 in Mt/year in 2010 and accounted for 50% of total production (1,400,000 Mt / year) (GAFRD, 2010).

Giant freshwater prawn *M. rosenbergii* has become with Nile tilapia an important part of polyculture ecosystem in developing countries due to large size attainment, tolerance to water quality changes, ability to cope with handling stress and ability to feed on unconventional feeds (Hossain and Islam, 2006). The Egyptian annual production of freshwater prawn has significantly increased from 600 Mt in 1995 to 5,409 Mt in 2010 (FAO, 2010).

Both Nile tilapia and freshwater prawn prefer similar temperature ranges, so far are not plagued with major disease problems, and reach market size in Egypt within 6 months of culture (Goda, 2008). Tilapia and prawns have different food and feeding habits. Tidwell *et al*. (1995) reported that, freshwater prawns feed on benthic organisms, detritus and feces (Zimmermann and New, 2000). Tilapia is regarded as an omnivorous species and capable of feeding on benthic and attached algal and detritus aggregates (Azim *et al*., 2003).

1.1. Nile Tilapia (*Oreochromis niloticus*)

1.1.1. Classification

Phylum : Chordata
Subphylum : Vertebrate
Super class : Osteichthyes
Class : Actinopterygii
Subclass : Neopterygii
Infraclass : Teleostei
Super order : Acanthopterygii
Order : Perciformes
Suborder : Labroidei
Family : Cichlidae
Genus : *Oreochromis*

Species : *niloticus* (Nandlal & Pickering, 2004)

1.1.2. Water Quality and Environmental Conditions

Water quality is one of the most important factors affecting the success of aquaculture production. If water quality is good, then good results can be achieved easily. In fish culture, water quality should be managed to regulate environmental conditions so that they are within the desirable range for survival, growth and reproduction of fish (Nandlal and Pickering, 2004; Shehata *et al.*, 1995). Hence, the water quality (physical and chemical) led within the permissible levels required for optimum growth and development of aquatic animals (Abdel-Hakim and Ammar, 2005). The main factors that influence growth of tilapia under environmental conditions of culture include water quality factors like temperature, salinity, dissolved oxygen, and pH value (acidity).

1.1.2.1. Temperature

Shepherd and Bromage (1992) reported that water temperature is an important factor for controlling rate of growth. Under practical conditions of fluctuating water temperature, the fish farmer should endeavor to plan his policy so that peak temperatures coincide with the later part of the main growing cycle, because the growth rate increase with an increase in temperature up to the optimum and down to reach the upper lethal temperature.

Thermal limits of *O. niloticus* are narrower for early stages and reduced survival of embryos and juveniles can occur at temperatures that are within the tolerance range for adult (Cossins and Bowler, 1987). Lethal limits, upper and lower temperatures that are fatal for all individuals, vary for each species (Phililppart and Ruwet, 1980).

Nile tilapia is described as having a high tolerance for a range of temperatures, with a lower lethal limit of 11-12 ºC and an upper limit of 42 ºC, and the preferred temperature is between 31-36 °C (FAO, 2006; Fish Base, 2007).

Most tilapia do not eat or grow at water temperature below 15°C and do not spawn at temperature below 20°C (Bradach *et al.*, 1972; Chervinski, 1982; Nandlal and Pickering, 2004).

1.1.2.2. Salinity

Salinity is known to exert selective pressure on studied fish developmental stages, influencing reproduction, dispersal and larval recruitment in marine, coastal and estuarine habitats (Anger, 2003).

Tolerance to salinity was assessed by correlated observations on gill structure, plasma sodium levels and gill Na^+/K^+ ATPase activity. All fish presented a gill epithelium structure characteristic: chloride cells (CC) on the lamellae and the filaments, when an increase in external salinity induced the proliferation of CC on filaments. Avella *et al.* (2005) reported that fresh water fish don't have chloride cells (CC) on the lamellae and few CC on the filaments. Tilapia is much more tolerant of salt water than most other freshwater fish. Adult Nile tilapia can tolerate salinity of up to 30‰ if the salinity increases gradually so they have time to get used to it (Nandlal and Pickering, 2004). Fry and small juveniles are happiest at less than 10‰ and will all die if salinity goes above 14 ‰ (Nandlal and Pickering, 2004). Eggs were able to withstand elevated rearing salinities up to 20‰, but transfer to 25‰ induced 100% mortality by 48 h post-fertilization (Fridman *et al.*, 2012).

Most tilapia species are considered euryhaline, in other words, they are capable of withstanding a wide range in salinity (Phililppart

and Ruwet, 1980). Al Asgah and Younis (2006) reported that *O. niloticus* is considered less euryhaline than most species, but it can tolerate the water salinities up to 18‰ and the upper threshold of the optimum salinity for optimum growth is 6‰.

Moharram *et al.* (1999) found that, the hepato-somatic and gonado-somatic index increased with increasing salinity for *Oreochromis* species and they are recommended to culture in the brackish water (17.8‰).

Assem (1995) indicated that, the optimum salinity produces the highest rate of growth was 20‰. In general, *O. niloticus* exists in a variety of freshwater and brackish habitats in the shallow water (FishBase, 2007).

1.1.2.3. pH values

The pH of the water is a measure of acidity (the hydrogen ion content in the water). Tilapia can survive in a wide range of water pH (Nandlal and Pickering, 2004).

Zweig *et al.* (1999) and Teichert-Coddington and Green (1993) recommended that, pH for good growth of tilapia is 6.5–9. Acidic water (with a low pH) will not support the growth of the phytoplankton, zooplankton and detritus-digesting bacteria that are important for fish growth. Nandlal and Pickering, (2004) reported that tilapia can tolerate pH of 5.0–10.5 without dying, although such extreme values are not good for growth, mostly die with a lower pH 4, also it does not spawn at pH 4.0-5.0 (Zweig *et al.,* 1999).

1.1.2.4. Dissolved Oxygen

Tilapia can survive in extremely low oxygen levels but will not grow well under such conditions (Jobling, 1994). Oxygen dissolves

into pond water from the air, slowly, phytoplankton in the water, during the daytime, by addition of new water, and by rain water splashing onto the water's surface. The dissolved oxygen (DO) limit for *O. niloticus* has been found to be 0.01 parts per million (ppm) (Nandlal and Pickering, 2004).

Mires (1983a) showed that tilapia can tolerate low oxygen concentration (down 3mg /L), whereas, Ross and Ross (1983) indicated that tilapia *O. niloticus* is an efficient respiratory regulator in water containing dissolved oxygen above 3mg /L.

For good growth, the oxygen level should ideally be above 3mg/L in the daytime; though as low as 1mg/L measured in the early morning has been found acceptable, and this was correspond with Broussard (1985), he pointed that, tilapia need about 5mg /L of oxygen or more to avoid stressful conditions. Likewise, Sweilum (1995) found that, the oxygen content for best growth and reproduction of Nile tilapia reared in earthen ponds ranged from 8.0 to 8.3 mg/L.

1.1.2.5. Photoperiodic Manipulation

Photoperiodic manipulation is emerging as an acceptable approach of practical application for regulating physiological functions in fish farming. Photoperiod techniques have been found to be influential on many physiological functions in fish species such as growth, reproduction and gonadal maturation, and are now widely used in aquaculture to alter spawning season, manipulate maturation and stimulate growth (Biswas and Takeuchi, 2002; Gines *et al.*, 2003).

Growth stimulating long photoperiod treatments either improve food-processing efficiency directly or suppress sexual maturation thus redirecting energy from gonadal development to somatic growth (Boeuf and Le Bail, 1999; Gines *et al.*, 2003). Biswas

et al. (2005) suggest that reproductive activities in *O. niloticus* (230–340 g) can be arrested by photoperiod manipulation and fish exposed to 6L: 6D photoperiod could not successfully spawn after three to four spawning cycle. Rad *et al.* (2006) obtained support the idea that continuous artificial lighting may be influential on enhancing somatic growth and delaying gonadal development in Nile tilapia during fingerling stage.

Moreover, photoperiodic manipulations have been found to influence metabolic rate and growth of Nile tilapia through energy conservation and stimulated food intake (Biswas and Takeuchi, 2003; El- Sayed and Kawanna, 2004).

1.1.3. Feeding habitat

Nile tilapia is a tropical species that prefers to live in shallow water. In the wild, tilapias are generally omnivores, feeding on plankton (phytoplankton, zooplankton), benthic organisms, periphyton, detritus, small fish, and aquatic plants (FAO, 2006; 2007). Specifically, adult of Nile tilapia feeds on eggs, larva, and other young fish, thus affecting the biological cycle of native species, while, Fry are feeding on aquatic and terrestrial insects, and aquatic larvae. As they grow, they begin to eat more and more phytoplankton until it becomes their primary source of food (Trewavas, 1983).

Moriarty and Moriarty (1973) pointed out to the ability of this species to digest blue-green algae. According to Spataru (1982) *O. niloticus* feeds on diatoms, protozoa and invertebrates. Sweilum (1995) added that the natural food organisms recorded in the alimentary canals of carp and tilapia were chrysophyta, chlorophyta, cyanophyta (phytoplankton); cilliata, rotefera, cladocera, copepoda

(zooplankton), and chironomidae, oligochaeta (bottom funa) with different percentage composition and frequency occurrence.

Nile tilapia can filter feed by entrapping suspended particles, including phytoplankton and bacteria, on mucous in the buccal cavity, although its main source of nutrition is obtained by surface grazing on periphyton mats (FAO, 2006; 2007). In captivity, tilapia readily accept artificial diets such as powder mash, crumbled pellets and pelleted feeds, if sized appropriately to fit into their mouth. This means that the fry and fingerling stages need plankton, or mash, rather than a pellet. Chicken manure is often an important component of food production for fry and fingerlings. Manure stimulates phytoplankton production, and also bacteria production on the bottom of the pond (detritus). The phytoplankton is food for zooplankton. Also phytoplankton, through photosynthesis, is the chief producer of dissolved oxygen in the pond, which is used by all organisms including fish. A combination of rice pollard (50%) and fish meal (50%) is commonly used as fry and fingerling feed (Nandlal and Pickering, 2004).

Al Sedfy (1990) cited that, experiments were conducted in glass aquaria to determine the occurrence of cannibalism among different size groups of Nile tilapia fry and fingerlings. There was an increasing occurrence of cannibalism as the tilapia fingerlings paired with the fry increased in size. El-Husseiny *et al.* (2004) mentioned that, the optimal feeding schedule of fish is important for their efficient production. They indicated that, the optimal feeding regime for tilapia was either once at 07:00 pm or three times at 07:00 am, 01:00 pm and 07:00 pm. Bahnasaway *et al.* (2003) demonstrated that, 50% satiation feeding is an effective feeding rate for improving body composition

and production of Nile tilapia cultured in fertilized freshwater earth pond.

1.1.4. Reproduction and Spawning

Nile tilapia has morphological differences between the mature the male and female. The male has two ventral body openings (anus and urethra), but female has three ventral body openings (anus, oviduct and urethra), and they have coloration under the jaw reddish in males and grayish in females. Sexual maturity in ponds is reached at an age of 5-6 months (Nandlal and Pickering, 2004; FAO, 2006, 2007).

Male tilapias are usually larger than females of the same age because the males grow twice as fast (FAO, 2006, 2007). Early and uncontrolled reproduction of the young fish causes overcrowding and stunted growth in pond cultivation. All that, led to the culture of monosex, preferably all male populations, it is important for the production of higher yields and more uniformly sized fish (Zaghloul *et al.*, 2002). The fecundity and maturity stages of different fish species including tilapia were also affected by environmental conditions (temperature, light and wind) and geographical locations (tropical and subtropical regions) (Ray, 1978; Lam, 1983; Duponchelle and Legendre, 2001; Farag, 2003).

The different environmental locations in Egypt are effects on the reproductive performance. Elghobashy (2000) noticed that, Abbassa stocks were the highest in fry production when compared with Zawia and Maryout strain at the first and second spawning seasons. Therefore, he recommended to use Abbassa stock or any cross with it. The temperature plays at important role in the spawning seasons. In Egyptian Delta, spawning of Tilapia may extend from April to

November with a maximum spawning in early summer (El-Saby, 1951). Rising temperature to 20° C or higher triggers the spawning operations of Tilapia, which start in April or May (Mires, 1983b). Farag (2003) exposed *O. niloticus* to different levels of water temperature ranging from 20° C to 36° C during the three spawning seasons in Sedy Salm City, Kafer Al-Sheikh. He found that the optimal reproductive performance occurs through summer seasons during May and the optimal water temperature was 28-31° C.

That is related between the feeding and spawning of Nile tilapia. According to Zaghloul *et al.* (2002) larger fish have greater fecundities, so the increase in growth should also increase egg numbers. They found that, the best size of female for breeding is 150–300g. On average a 200g breeder would produce 200–500 fry per month. Essa (1995) has been studied the relationship between the feeding and reproduction of *O. niloticus*. Shortly, after ovulation a fat and carbohydrate rich diet should be used to accelerate the egg development. After the completed vitellogenesis the females need more protein rich food, less fat and carbohydrate for not only building-up of sexual products but also to prevent unnatural fatness of the ovaries. When the females fed with diet rich in fat, carbohydrate and protein all the time, this diet causes fattening of the ovaries, and thus reduces normal process of reproduction. Hassouna *et al.* (2002) carried out of the effect of different crude protein levels (25, 30, 35 and 40%) of commercial diets on reproductive and growth performance of Nile tilapia. They found that, 35% crude protein was the best among the tested commercial diets followed by the 30% crude protein one. Gunasekera *et al.* (1995) found that tilapia fish fed diets of higher protein level (32 and 40%) exhibit better fecundity than those fed

lower protein (17 and 25%). Similarly, Siddiqui *et al.* (1998) reported that, the average number of females spawning and eggs per spawn in tilapia species were increased with increasing the dietary protein level to 40 and 45%.

Both vitamin C and vitamin E have specific role in reproduction of teleosts. Mohamed *et al.* (2003) studied effect of alpha-tocopherol acetate (vitamin E) on fecundity and reproductive physiology of *O. niloticus*, it uses as proved to be a reproductive promoter. Their results confirm the essentiality of vitamin E supplementation in producing spawn with better egg and larval quality. On the other hand, Soliman *et al.* (1986) indicated that, impaired fecundity, hatchability and fry survival were observed in eggs originating from females of tilapia provided with diets containing low amounts of ascorbic acid (vitamin C).

1.1.5. Nutritional requirements

Recently, an increased interest in aquaculture has revealed of knowledge concerning nutritional requirements of fish although they have many of the dietary requirements as warm blooded species (Eid *et al.*, 2003). Addition of artificial feeds plays an important role especially under conditions of heavy stocking, when natural feed supply has declined or completely disappeared. The feed dietary in aquaculture should be rich in protein, carbohydrate, and fats, and should contain vitamins, minerals and growth promoter substances to be physiologically balanced (Huisman *et al.*, 1979).

1.1.5.1. Protein requirement

Protein is the main constituent of fish body thus sufficient dietary supply is needed for rapid growth. Dietary protein is an

important aspect in achieving efficient fish production and its needs should accommodate fish requirements due to age/weight (Abdel-Tawwab *et al.*, 2010). Lovell (1989) noticed that, the level of dietary protein producing maximum growth of tilapia depends upon the protein quality, energy content of the diet, the physiological state of the fish, age, reproductive state, and the environmental factors such as temperature, salinity…. etc. So, the amount of protein in the diet should be just enough for growth and tissue repair because it is more expensive than carbohydrates and fat, especially, when use the fish meal as the protein source (Khattab, 2001; Lovell, 1980a).

Research investigations on tilapia nutrition have focused on determination of the minimum level of protein requirements, where it was estimated that protein requirements tilapia has ranged between 20 and 56 % CP (Shiau *et al.*, 1987; El-Dahhar, 1994; El-Tawil, 1998). Specifically, the optimum protein level of the diet can be lowered if the energy level is increased, due to protein-sparing action of energy containing nutrient sources (Shiau and Peng, 1993). El-Ebiary and Zaki (1999) suggested that, the best growth rate, survival percentage and FCR for juvenile's *O. niloticus* was obtained at dietary protein level from 30 to 40%. El-Dhhar *et al*. (1999) recommended 26% dietary protein for the best growth and immune (resistance to *Aeromonas hydrophila* infection). El-Nady *et al.* (2001) and Eid *et al*. (2003) indicated that, the optimum dietary protein level for Nile tilapia (weighing 20g) was 30%, but the best growth of tilapia with 25-30% protein level diets. Khattab *et al.* (2001) studied effect of stocking density (15 and 30 fish/100), and level of protein (25, 35, 45) on the growth performance of *O. niloticus*. The maximum growth was obtained with 45% CP at low density and 35% CP, whereas the lowest

growth with 25% CP at high density. Abdel-Tawwab *et al.* (2010) fed Nile tilapia on diets containing 25, 35, and 45% crude protein. They found that, the best growth rate of fry tilapia was obtained at 45% CP, while fingerling and advanced juvenile showed optimum growth performance with the 35% CP diet.

1.1.5.2. Amino acid requirement

Amino acids and proteins are critical molecules because of the role they play in the structure and metabolism of all living organisms. Fish can't synthesize all amino acids and must acquire several in their diet, thus, can be divided amino acids into two groups, essential and non-essential (NRC, 2011). An optimized amino acid profile can only be obtained by combining a number of plant protein ingredients as no single agricultural crop can provide a suitable amino acid composition (Gaylord *et al*., 2010). Hence, soybean and yeast almost have the best of the essential amino acid composition (Wu and Jan, 1977). Nile tilapia requires 10 essential amino acids (Table 1) namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine.

Deficiency in EAAs will result in reduced growth performance and feed utilization (Zhou *et al*., 2007). Specifically, Tacon (1992) indicated that, arginine deficiency in common carp lead to reduced growth rate, increased mortality and incidence of lordosis. Because It is involved in nitrogen metabolism, polyamine synthesis and production of nitric oxide; beside, the synthesis of creatine and a major reserve of high-energy phosphate for ATP (adenosine triphosphate) regeneration in the muscle (Denis *et al*., 1998). Therefore, the knowledge of amino acid requirements would be useful in formulating balanced nutrients in feeds (Zhou *et al*., 2007).

Table 1. Essential amino acid requirements for growth of young Nile tilapia (g/100 g Protein)

Essential amino acid (EAA)	EAA Requirement (g/100 gprotein)	
	Santiago and Lovell (1988)	**NRC (1993)**
Arginine	**4.20**	**4.1**
Histidine	**1.72**	**1.7**
Isoleucine	**3.11**	**3.1**
Leucine	**3.39**	**3.4**
Lysine	**5.12**	**4.6**
Methionine	**2.68**	**-**
Methionine plus cystine[1]	**3.21**	**3.2**
Phenylalanine	**3.75**	**-**
Phenylalanine plus tyrosine[2]	**5.54**	**5.6**
Threonine	**3.75**	**3.8**
Tryptophan	**1.00**	**1.0**
Valine	**2.80**	**2.8**

[1]Cystine included as 0.54% of the protein.
[2]Tyrosine included as 1.79% of the protein.

1.1.5.3. Lipid requirement

Lipids are important component of diet, for both energy and essential fatty acids sources. Essential fatty acid (EFA) requirements vary qualitatively and quantitatively with both species and during ontogeny of fish, with early developmental stages and bloodstocks being critical periods (Tocher, 2010). The fatty acid composition of the cell membrane is especially important in disease resistance because many of the mechanisms and reactions are membrane-associated (Shehata and Goda, 2000).

Environment and/ or trophic level are major factors, with freshwater species generally requiring C18 polyunsaturated fatty acids (PUFA). All vertebrates have an absolute dietary requirement for certain, specific C18 polyunsaturated fatty acids (PUFA). If a dietary deficiency occurs, the animal stops growing and reproducing, it develops various pathologies and eventually dies (Tocher, 2010; Das, 2006), and also, it plays a role in fish immunity (Fracalossi and Lovell, 1994). Takeuchi *et al.* (1983) found that, *O. nilotica* need during ontogeny to 0.5 (% dry diet) of C18 EFA. Hassanen (1988), Moharram *et al*. (1999) and Shehata and Goda (2000) indicated to 6% of lipid seemed the optimum one for gonad maturation of both sexes and functioning of humoral immune response of *O. niloticus*. But, Gaber (2000) found the best fat content of Nile tilapia flesh was 12mg of clove oil as lipid source and led to increasing fish growth.

1.1.5.4. Carbohydrates requirement

Carbohydrates are the least expensive form of dietary energy for humans and domestic animals, but utilization by fish varies and is lower than that of domestic animals (Furuichi and Yone, 1981). Tilapias were found to utilize complex carbohydrates such as dextrin or starch for growth more readily than simple sugars such as glucose (Tung and Shiau, 1991).

There were differentiations among carbohydrate sources for growth performances. El-Saidy and Gaber (1999) used different dietary carbohydrate sources; corn meal, wheat bran, starch, date palm and akalona. They found the highest final individual weight and the best protein and fat contents were achieved in case of fish fed on wheat bran and corn meal. Gaber (2002) noticed that, the diet of Nile tilapia containing carbohydrate (starch) to lipid (soybean oil) ratio of 4.8:1 showed significantly lower performance, while 2.89:1 due higher growth performance and physiological fuel values.

Niacin is a vital part of the coenzymes needed to release energy from carbohydrate. Shiau and Suen (1992) were conducted to determine the adequate dietary niacin levels for juvenile hybrid tilapia, when diets containing 38% glucose or 38% dextrin (from corn) as the carbohydrate source were fed. They indicated that best growth was 26 mg/kg of glucose and 121 mg/kg of dextrin.

1.1.5.5. Vitamin requirement

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 (NRC, 1993).

Watanabe (1995) mentioned that, vitamin requirements for fish vary with species, size and environmental conditions.

Vitamin C (L-ascorbic acid; ASC) is probably the most commonly used vitamin. It is highly soluble in water and functions as an effective reductant. It also plays a role as a coenzyme of oxidation enzymes. The fish depend upon exogenous source of vitamin C as they cannot synthesize it (Chatterjee, 1978; Kojo, 2004). Vitamin C is involved in the metabolism of neurotransmitters, lipids, and collagen. The National Research Council, NRC (1991) indicated that, vitamin C requirement for tilapia species is 50µ / Kg. diet. Whereas, 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*).

The reduction vitamin E (α-tocopherol) causes reduce of growth, skin and fin hemorrhage and anorexia (Roem *et al*., 1990). In all animals including fish, it 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.

1.1.5.6. Minerals requirement

The fish uses inorganic elements to maintain osmotic balance between fluids in the fish body and water (Lovell, 1980b). The most important of minerals for fish is calcium, phosphorus, copper, and iron. Robinson *et al*. (1987) reported a requirement for tilapia as 0.5 % calcium for normal bone mineralization and no pathological signs of phosphorus deficiency were recorded. Copper promotes iron absorption from the gastrointestinal system, and is involved in the transport of iron from tissues into plasma (Sorensen, 1990). Ayyat *et al*. (2000) fed Nile tilapia on diet with two levels of copper (0.5, 100 mg/kg diet) as supplement, and they found, the increase in dietary copper level was accompanied with the increase in fish body weight at different experimental periods.

Fish acquire iron predominantly from the diet with negligible iron uptake from the gills; EL-Serafy *et al*. (2007) added different iron levels to diet was 1200 mg Fe^{+}|kg diet from ferrous sulphate improves the growth performance and hematological parameters of *O. niloticus* fingerlings.

1.1.5.7. Protein energy ratio requirements

Fish obtains the energy required from feed or, in periods when deprived of feed, from the body stores. A dietary excess or deficiency of useful energy can reduce growth rate, because energy is needed for maintenance and voluntary activity. The diet containing excess energy can restrict food

consumption and thus prevent the intake of necessary amounts of protein and other nutrients for maximum growth (Hepher, 1988).

Chow and Halver, (1980) reported that omnivorous fish can utilize carbohydrate as a source of energy more than carnivorous fish. Although, the availability of carbohydrate calories for energy is very low of Nile tilapia and consequently some part of the dietary protein is consumed as energy. Addition of lipid as energy source to the diet is helpful an effective utilization dietary protein (Hassanen, 1988). The more protein is used for energy with inadequate dietary energy levels, Siddiqui *et al.* (1988) indicated that, the optimum protein energy ratio for Nile tilapia ranged from 70 to 75 for average weight 30g; likewise, Eglal (1994) showed the best 106 for O. *niloticus* average weight 0.40g.

1.1.5.8. Digestion trails

Formulation of well-balanced diets and their adequate feeding are the most important for successful aquaculture. The nutritive value of formulated feeds depends on the digestibility of the individual components. Metabolic responses to various components of the diet are important determinants of the digestive physiology and may provide an index of the efficiency of nutrient utilization. Thus, the first task in evaluating the potential of any foodstuff for inclusion in a diet is the measurement of its digestibility (González-Penã *et al*., 2002). The apparent digestibility coefficient (ADC) is an important parameter not only to evaluate the nutritional quality of a food source, but also to what degree it is assimilated by the animal (Oliveira *et al*., 2008).

Hanely (1987), El-Sayed and Toshima (1992) and Goda (2002) were concluded to evaluate the apparent protein digestibility and amino acid availabilities of fish meal replaced with soybean meal and full fat soybean O. *niloticus*. They found the diet containing either soybean meal or full fat

soybean had the highest value for protein and essential of amino acid at replaced 50%. EL-Saidy and Gaber (1999) found the highest ADC of crude protein, crude fat and energy of Nile tilapia was with wheat bran and corn meal diets and the lowest was observed with akalona diet, and but El-Saidy (1999) did not observed difference between control and other groups of fish fed cottonseed meal at partial and total replacement of fish meal, while Khattab (2001) noticed that the highest ADC carbohydrate and gross energy were with fish groups of *O. niloticus* fed black seed cake diets as carbohydrate source.

Ayyat *et al*. (2000) were obtained that addition of copper to diet of Nile tilapia were improved the ADC of crude protein, crude fat, carbohydrate and energy. El-Saidy and Gaber (2004) found the ADC of protein and lipid of *O. niloticus* were relatively high for most treated diets with Yucca (*Yucca shidigera*), whereas EL-Saidy (2008) observed that the fat digestibility of Nile tilapia was not different among diet replaced of fishmeal with cow pea seed meal except 75% and 100%. Azaza *et al*. (2009) have evaluated the apparent dry matter digestibility and protein of faba beans as a replacement for soybean meal in practical diets of juvenile *O. niloticus.* Apparent dry matter digestibility reduced with increasing faba bean meal levels. While, apparent protein digestibility decreases in fish fed 36% of faba bean meal. Lund *et al*. (2011) repoted the nutrient utilization in organic trout *Oncorhynchus mykiss* diet of fish meal replaced with a fixed matrix of organic pea, horsebean and rapeseed plant protein concentrates. Substituting fish meal with organic plant protein concentrates increased the ADC of protein and lipid at the highest level.

1.1.6. Histological studies

1.1.6.1. Liver and pancreas

The liver of the Nile tilapia is a non-lobulated organ located in the pectoral region of the peritoneal cavity of the fish body. The hepatocytes are

slightly rounded or oval, and rounded centrally located nucleus. They are arranged in a tubular pattern the tubules of which are composed, each of six to ten cells surrounding a minute sinusoid. The liver contains diffused areas of exocrine pancreatic tissue that are encircled by a thin layer of connective tissue capsule and in the centre of the pancreatic structure became obvious one or more blood vessels occur) Mohamed, 2009).

Herbicide and heavy metal pollution severely affects aquatic organisms at higher trophic levels including human beings. All these pollutions cause severe damage into tissues of aquatic animals in aquaculture. Jiraungkoorskul *et al.* (2003) and Peebua *et al.* (2008) exposed *O. niloticus* to two types of herbicide (glyphosate and subchronic alachlor, and that effects vacuolation and hydropic swelling of hepatocytes and nuclear pyknosis. Hemmaid and Kaldas (1994), Abdelmeguid *et al.* (1999), Abd El-Gawad (1999) and Figueiredo-Fernandes *et al.* (2007) carried of the effect of heavy metals accumulation in the liver of Nile tilapia with different concentration. Mohamed (2003) studied on the health condition of *O. niloticus* living in the polluted water of El-Salam canal in Egypt.

Tuan *et al.* (2002) added aflatoxin B1 (AFB) to diet of Nile tilapia at several concentrates. At 10 mg AFB|kg diet found excess lipofusion and irregularly sized hepatocellular nuclei, while at 100 mg|kg caused severe hepatic necrosis and 60% of fish in this treatment died. Valentim-Zabott *et al.* (2008) were fed *O. niloticus* on Homeopatila RS diet (vital energy of the organism) for treatment and prevention against diseases. It caused less hepatic lipid inclusions. Other study fed tilapia on copper-loaded diet and the results expanded fatty change in the liver (Shaw and Handy, 2006).

1.1.6.2. Gonads

The ovaries of the Nile tilapia are yellow paired elongated structures, lying dorsal to the alimentary canal and ventral to the swim bladder, and extend

in the abdominal cavity from the posterior to the anterior. On the other hand, the germinal epithelium projects into the ovary as numerous folds, the ovarian lamella, are containing different stages of many oocytes. While the testes are white yellowish elongated organs, located in the body cavity ventral to the kidneys and extend anterior to the beginning of the cardiac stomach. The testis is a compound tubular gland surrounded with a dense thick connective tissue capsule, the tunica albuginea, and composed of a large number of seminiferous tubules (Mohamed, 2009).

Shalloof and Salama (2008) investigated the seasonal ovarian development of the freshwater Nile tilapia in the Abu-Zabal Lake, Egypt. They studied stages of early previtellogenic oocytes, vitellogenic oocytes. Some studies reported about effects some vitamins and supplements diet on the histology of gonad. Celik and Altun (2009) observed that vitamin E levels increased growth stages of the ovarian and testis tissues. Thus, Mahmoud (2009) was noticed that, the promoters (vitamin E, C and cobalt chloride) alleviated of the pollution effect in gonad of *O. niloticus*, because the pollution causes several histopathological changes on the gonads of *O. niloticus* and *Tilapia zillii* (Mohamed, 2003; Mohamed, 2009). Jegede and Fagbendro (2008a) added pawpaw seed meal (fertility control agents) to a basal diet of *O. niloticus* at several concentrations, and the results showed some morphological and histological changes of the gonads. Whereat, they found color change of gonads, and were noticed necrosis and increased of interstitial cells, disintegration of sperm cells, and trophy of seminiferous tubules; in the other hand, the ovary has had necrosis and severe atretic follicle. In addition, Jegede and Fagbendro (2008b) carried out effect of neem (*Azadirachta indica*) leaf meal (NLM) (anti-bacterial, anti-fungal, anti-viral and anti-fertility properties) on the histological gonads of *Tilapia zillii*; they were observed destructive of testes and ovaries tissues. Moharram and Ebiary (2003) fed of

Nile tilapia on diet containing 60µg|g of 17α-ethynyltestosterone (androgen treatment) with active yeast, which caused to destructive of histological gonad development appearance and have been observed histological anomalies of the gonads for both sexes such as pyknosis and necrosis.

1.2. Freshwater Prawn (*Macrobracium rosenbergii*)

1.2.1. Classification

Phylum : Arthropoda
Subphylum : Mandibulata
Class : Crustacea
Subclass : Malacostraca
Series : Eumalacostraca
Super order : Eucarida
Order : Decaopoda
Suborder : Natantia
Infra order : Caridea
Super family : palaemonidea
Family : Palaemonidae
Genus : *Macrobracium*
Species : *rosenbergii* (Edwin *et al*. 1993; FAO, 2005)

1.2.2. Water Quality and Environmental Conditions

There are many environmental factors effects on the energy budget and production of this species (Du and Niu, 2002). Many farmers were aware of the environmental effects of current systems and attributed multiple problems to external pollution. The major problems most commonly identified by respondents were seed supply (67%), disease outbreak (64%), and external pollution (37%). Approximately one third of respondents cited low production that could be caused by a number of unknown factors (Schwantes *et al.*, 2009).

1.2.2.1. Temperature

The temperature is major effect on the embryonic development, hatching period and all phase's life cycle of *M. rosenbergii* (Anger, 2001). Embryonic development rates, rapid larval length and faster hatching of *M.*

rosenbergii increased with increasing temperature except above 36°C, all mortality after 192 h (Manush *et al*., 2006). Herrera *et al.* (1998) were acclimating post larvae and juveniles of *M. rosenbergii* with temperature ranged from 20 to 32°C, results in critical thermal maxima from 37 to 41° C. Hsieh *et al*. (1989) suggested that the optimum water temperature ranged from 26 to 31° C, while below 24° C and above 35° C causes retarded development or mortality; Lal *et al*. (2012) revealed that the temperature tolerance investigations range of 30 ± 0.5°C produced optimal survival and growth. Generally, the temperature optima for the majority of *Macrobrachium* species appear largely similar, ranging between 20 and 30°C (Subramanian *et al*., 1980). In wild, Rao (1991) found that peak spawning of *M. rosenbergii* populations occurs between 29 and 30.5°C.

1.2.2.2. Salinity

Under culture, variations in salinity may break homeostasis and lead to significant stress in freshwater giant prawn, penaeid shrimp (Lee and Wickins, 1992). There are three prominent problems in long-term low salinity cultivation: slow growth, low survival rate (Li *et al*., 2007), and high susceptibility to pathogens (Wang and Chen, 2006).

M. rosenbergii experiences variations in environmental salinity at several stages of its life cycle. Although most populations inhabit fresh- water, the larval stage is spent entirely in brackish water (10 - 15 ‰). After metamorphosis the post-larvae begin to enter freshwater, a transition that may last for several weeks. Finally, the spawning migration from freshwater into brackish water by the adult females will expose them to large fluctuations in environmental salinity (Ling and Merican 1961; Daniels *et al*., 2000). The fresh water giant prawn is the hardiest and resistant species within the genera of *Macrobrachium* (0 to 24‰), and it can easily tolerate different salinities of water from fresh- to saltwater (Armstrong *et al.,* 1981; Schwantes *et al.*, 2009).

This characteristic called euryhaline and have most genus *Macrobrachium* (Short, 2004 ; Lal *et al*., 2012). In addition, M. *rosenbergii* maintained at salinities of 17.5 and 28‰ (Taylor and Funge-Smith, 1994). Generally, *M. rosenbergii* is indigenous to fresh and brackish water, and that are desirable for shrimp culture (abdelRazek *et al*., 1998), thus water salinity of 3 - 4 ‰ may be acceptable for the culture of *M. rosenbergii* (Daniels *et al*., 2000).

1.2.2.3. pH values

The optimal pH range for the growth of *M. rosenbergii* is 7.0–8.5 (Boyd, 1990). It is considered more sensitive to low pH as compared to other decapods crustaceans. Allan and Maguire (1992) suggested that the minimum acceptable level of pH was 6.2 based on the growth of prawns in different pH levels after 42 days. Molting of decapods crustaceans is affected by extrinsic factors such as temperature, salinity, light intensity, and pollutants and by intrinsic factors such as nutritional state and hormones (Kleinholz, 1985). The molting frequency of *M. rosenbergii* placed in pH 6.8, 6.2 and 5.6 was significantly lower than pH 7.4 and 8.2 (Brown *et al*., 1991).

1.2.2.4. Dissolved Oxygen

In prawn culture, dissolved oxygen (DO) demand is easy to meet by photosynthesis and the exchange of O_2 across the air water interface (Boyd, 1998). DO has often been considered an important environmental factor determining the success and intensification of prawn culture, and mostly, DO values have been recommended for intensive culture practice higher than 5 mg /L (Cheng *et al*., 2001). Low DO adversity affects the behavior and normal physiology of crustaceans, such as the survival, respiration and circulation, feeding, metabolism, growth, and molting of penaeid shrimps (AQUACOP, 1990; Li *et al*., 2006). Seidman and Lawrence (1985) indicated that DO concentrations below 2 mg l−1 significantly reduce the growth rate of *Penaeus*

vannamei and *P. monodon*; and this is similar with *M. rosenbergii*, it is stressed when DO falls below 2 mg O_2/L, and the lethal DO level for the prawns was found to be 0.5 mg O_2/L (Avault, 1986). On other hand, Rosas *et al*. (1997) have reported critical levels between 4.5 and 5 mg l−1 for *P. setiferus* and *P. schmitti* PL. About fresh water prawn, the optimal DO is almost 3.7 mg O_2/L (Boyd and Zimmermann, 2000).

1.2.3. Feeding habitat

Food and feeding is one of the most important factors affecting the growth as well as the yield, feed conversion and carcass composition of the prawns (Caine, 1975). In the nature, the adults of genus *Macrobracium* are omnivorous, eat greedy and frequently on both plant and animal materials, such as Pieces of mollusca, crustaceans, cut up flesh and internal organs of fish, grains of rice, wheat, peas, beans, ground nuts, coconuts, and fruits, etc. When sufficiently hungry, it may even become cannibalistic. Food materials are located mainly by the sense of smell and touch, when searching for food the first and second pair of the thoracic legs which are chelate sweep about actively (Ling, 1969).

During the early stages, *M. rosenbergii* can't be easily acclimatized with the artificial feed, so supplementation of live food organisms (such as *Artemia* spp and *Brachionus plicatilis)* is necessary at the larval and early PL stages of fishes and shellfishes (Bengtson *et al*., 1991; Alam *et al*., 1995). The prawn is usually quiet during day time and stays at the bottom of waters without much active locomotion and tends to avoid strong illumination. They are active at nighttime, searching for food (Ling, 1969); therefore, *M. rosenbergii* classified as having a benthophicomnivore feeding habits based on analyses of forgot contents (D'Abramo and Sheen 1994; Tacon, 1996). At the other side, Hussein and Obuid-allah (1992) indicated that *Caridina*

niloticus (freshwater prawn) is detritivorous mechanism; whereas, it is a detritus feeder and feeds on material accumulating on the bottom.

1.2.4. Reproduction and Spawning

The salinity plays on larvae survival, salinity also be important to egg, embryo and larval development. Whereas, Egg-bearing females migrate downstream to estuaries (2-5‰) and other areas to hatch their eggs and larval development takes place in brackish water (12±2‰). After metamorphosis into post-larvae, they assume a more benthic life style and migrate upstream again where they grow to maturity (Ling, 1969; Yen and Bart, 2008).

In reared tanks, the times of spawning fresh water prawn depends on the temperature. When mature, male prawns are considerably larger than females. Females become reproductively mature within 6 months of age (Spotts, 1981; D'Abramo *et al.,* 1995). Adult males can be categorized into three morphotypes; each morphotype represents a different stage in the development of the adult males from small males (SM) through orange-claw (OC) males to blue claw (BC) males (Ra'anan, 1982; Karpuls *et al*., 1986). Ra'anan and Sagi (1985) observed that SM and BC males are more sexually active than OC males. During reproductive season, the male deposits sperm held in a gelatinous mass underneath the body of the female between her fourth pair of walking legs. Within a few hours after mating, eggs are released and fertilized by the sperm. The female then transfers the fertilized eggs to the underside of the abdomen (tail) in a "brood chamber" formed by swimming appendages. The eggs are aerated and cleaned by movement of these appendages and remain attached to the abdomen until they hatch. The bright yellow color of newly spawned eggs gradually changes to orange, then brown, and finally to grey about 2 to 3 days before hatching. The number of eggs produced at each spawn is directly proportional to the size of the female. Generally, Fecundity of *M. rosenbergii* is from 40,000 to 60,000 eggs (body

weight 100g). At a temperature of 28 ºC, the eggs hatch approximately 20-21 days after spawning. The hatched embryo passes through 11 larval stages, and they cannot survive in fresh water beyond 48 hours and must migrate to brackish water. After metamorphosis to PL, the prawns resemble miniature adults, and adapt again to freshwater (Ogasawara, 1984; D'Abramo *et al.*, 1995).

AbdelRazek (1993) reported that, the period beginning with June up to October was the most suitable for rearing *M. rosenbergii* in Egypt and other warm temperate region. Yen and Bart (2008) clearly shows that female of fresh water prawn bloodstocks reared in lower salinity (0-6 ‰) was larger, reproduced early, and produced more offspring than at higher salinity (12 ‰).

1.2.5. Nutritional requirements

Feed is often the major expense in pond production of freshwater prawn, representing as much as 40-60% of operating costs (D'Abramo and Sheen, 1991). Fresh water prawn life history is marked by changes in morphology and behavior, with a shift from planktonic herbivory to omnivory in late protozoea and the adoption of a benthic existence as PL. These ontogenetic events are accompanied by significant changes in metabolic rates and digestive enzyme activities (Lemos *et al*., 1999; 2000).

1.2.5.1. Protein requirement

In both natural and prepared diets, protein is the most abundant ingredient for shrimp playing a critical role for growth and development (Sudaryono *et al*., 1995). The main diet of *M. rosenbergii* is *Artemia* as nature protein source (Kovalenko *et al*., 2002; Nhan *et al*., 2010) and its very expensive, therefore any management that improve feeding efficiency is important in reducing feed costs.

Several studies suggested many live foods replace live *Artemia* by other live food sources. AbdelRazak *et al*. (1998) used natural organisms (oligochaete worms and insect larvae‘‘Chironomus”) as a shrimp meal and cheaper; they found that the best survival and growth rates for prawn fed on mixed diets (live food and artificial food) compared to artificial shrimp meal (control). Habashy *et al*. (2000) found that frozen insect larvae promote growth rate of *M. rosenbergii.* Habashy and Daba (2006) revealed that mosquito larvae a live food failed to improve the growth rate and survival of the freshwater prawn when added to *Artemia* because mosquito larvae in live form stay the most time at the surface of water.

Balazs and Ross (1976) added different source of protein (soybean and tuna meal, or of soybean, tuna and shrimp meal) with three protein levels (15, 25 and 35%) for diet of juvenile *M. rosenbergii*. They were obtained larger prawns with higher protein content (35%) from soybean and tuna meal. New *et al.* (1980) suggested that protein level of 25% or possibly less, produce acceptable results for *M. rosenbergii* culture. On other hand, Antiporda (1986) was determined of dietary protein requirement for growth performed of *M. rosenbergii* in using ratios (20, 25, 30, 35 and 40% crude protein levels) based on fish meal and shrimp meal as the main sources of protein; and noticed no significant differences in all variables considered. Leena *et al*. (1997) mentioned that animal protein sources (mussel meal, meat meal, squid meal, shrimp meal, fish meal, and earth worm meal) supported growth, molting frequency and survival in freshwater prawn compared to plant protein source such as various oil seed cakes. In addition, Teshima (2006), Kabir-Chowdhury *et al*. (2007) and Goda (2008) reported that plant protein has been found to be relatively poorly utilized in crustaceans in terms of growth in comparison to protein of animal origin.

1.2.5.2. Amino acid requirement

Proteins and amino acids are critical molecules because of the role they play in the structure and metabolism of all living organisms. Naturally, fish meal has been the most important feedstuff used as a source of protein in aquaculture feeds because of its essential amino acid composition and palatability (Davis *et al*., 2004). The quality of protein sources is expressed as the amount of essential amino acids in the crude protein. In spite of, this information is important, but is not sufficient for optimizing formulations because digestive utilization of amino acids is always lower than the analyzed amount (Sibbald, 1987). A reduced digestion limits the bioavailability of amino acids needed for protein synthesis and growth. Some of these inhibitors are in fact proteins that occur in the protein source used to formulate the diet. As an example, some plant seed meals contain proteinase inhibitors that affect the degree of protein hydrolysis by shrimp digestive enzymes, leading to poor growth (Garcia-Carreño, 1996). In addition, the ontogenetic variations of shrimp are accompanied by significant changes in metabolic rates and digestive enzyme activities (Lemos *et al*., 1999).

Deshimaru and Shegino (1972) mentioned that the admixture of certain protein sources together with additives would provide the special pattern of amino acid distribution similar to that of the prawn. Mitra *et al*. (2005) reported that freshwater prawns appear to require 10 essential amino acids which are methionine, arginine, histidine, isoleucine, leucine, lysine, valine, phenylalanine, threonine, and tryptophan, and it is similar with marine shrimp *Litopenaeus vannamei* (Terrazas-Fierro *et al*., 2010). Specifically, Methionine is the first limiting amino acid in most shrimp diets (Forster and Dominy, 2006). Richard *et al*. (2011) were reported of black tiger shrimp juvenile (*Penaeus monodon*) on the effects replacing fishmeal by different

levels of plant proteins on growth performances and nutrient utilization of shrimp and on the availability of dietary nitrogen and amino acids.

1.2.5.3. Lipid requirement

Highly unsaturated fatty acids (HUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), has recognized as very important nutrients for the growth of the crustaceans. Growth-enhancing responses to dietary oils containing comparatively high levels of HUFA (22:6 ω-3 and 20:4 ω-6) were observed for fresh water prawn (Sandifer and Joseph, 1976). The importance of HUFA has been concluded that *M. rosenbergii* have the ability to synthesize saturated and monounsaturated fatty acids from palmitic acid (Reigh, 1985). Like Artemia, Moinadoes not the requirement of the predator crustaceans with respect to EPA and DHA though it contains 60–70 % (dry weight) protein. Thus, there is a need to enhance the nutritional quality by enriching them with HUFA (Das *et al*., 2007).

Querijero *et al*. (1997) studied the utilization of mono-unsaturated fatty acid and found that HUFA are predominantly of the ω-6 series in early stage of prawn and of the ω-3 series for the rest of the larval stages. The fatty acids of the early stage of prawn are palmatic, oleic and linoleic acids, while, the most required fatty acids for the rest of the larval stages are palmitic, stearic, oleic, linolenic and eicosapentanoic acids. Therefore, Hsieh *et al*. (1989) indicated that fatty acid composition is more important than total lipid content. Dietary lipids play an important role as a source of energy and essential fatty acids. Cavalli *et al*. (2000) illustrated that effect of linoleic acid (ω-3) HUFA has great effect on the reproductive performance and offspring quality of prawn. Sheen and D'Abramo (1991) defined the optimal amount of dietary (1:1 cod liver oil/corn oil) required for *M. rosenbergii* from 2 to 10%. Tiwari and Sahu (1999) found that maximum weight gains of prawn where soya-lecithin was included at 5% along with 1% cod liver oil, grounded oil mixture

for rearing post-larvae of fresh water prawn. Whereas, Habashy (2003a) replaced of cod liver oil in the diet by sunflower oil, and obtained the best growth of *M. rosenbergii* PL at 5% along with 1% cod liver oil.

1.2.5.4. Carbohydrates requirement

Carbohydrate is the cheapest dietary energy. It is important for storage of dietary energy in the synthesis of chitin, steroids and fatty acids. Dietary carbohydrates such as cellulose facilitate the rate of passage of food thought the gut (Clifford and Bricks, 1983). Utilization of carbohydrates by shrimp appears to vary with complexity and processing of the carbohydrates (Chuang, 1991). With limited information on the bioavailability of energy from carbohydrate sources, the nutritional and economic value of these ingredients is difficult to evaluate properly (Zaki, 1999). In addition, the types and levels of carbohydrate in the diet have been shown to affect the growth and survival of prawn (Deshimaru and Yane, 1978; Abdel-Rahman *et al*., 1979). About fresh water prawn, the complex dietary polysaccharides are utilized better and yield higher growth rates than mono and disaccharides (Briggs, 1991).

Chaung *et al*. (1985) reported that freshwater prawn has α-amylase and cellulose activities in the hepatopancreas, stomach or intestine much higher than marine shrimp. Zaki (1999) used different source carbohydrates for diet of *M. rosenbergii*. He found that the unrestricted rice bran gave the highest growth performance and it is achieved the optimal economic value in feed.

1.2.5.5. Vitamin requirement

In shrimp nutrition adding certain amount of vitamins as supplement, it is required of the principal component of connective tissue, the organic substances of exoskeleton and the ground substances between cells, it is also required for the synthesis of collagen, and to promote healing. It is probable that tissue repairing and healing are involved in molting (Jaff, 1984). But, Castille and Lawrence (1989) found that elimination of vitamins from pellet

feeds did not affect the growth of shrimp, and suggested that vitamin supplementation is not necessary when natural foods are present in ponds.

The importance of vitamin C (ascorbic acid) and vitamin E (α-tocopherol) has been demonstrated for development and reproductive processes of aquatic animals. The major benefits of these vitamins are due to their capacity of scavenging reactive oxygen species in biological fluid and membranes. In shrimp, supplementation of vitamin C and E was reported to improve reproductive performance such as ovarian growth, higher egg hatchability, better spawning performance, fertilization and egg hatchability (Cahu *et al*., 1995; Du *et al*., 2006). Thus, Nguyen *et al*. (2012) are recommended to obtain higher egg hatchability and better quality larvae by supplementation of VC and VE in feeds for female shrimp during maturation. In addition, dietary vitamin C and E requirements for growth of early development stages in shrimp species (Koshio, 2010). Crustaceans fed diets deficient of vitamins especially vitamin C develop melanized lesions distributed throughout the collagenous tissue underlying the exoskeleton, abnormal colorization, and mortality (Shigueno and Itoh,1988). Cavalli *et al*. (2003) suggested that feeding *M. rosenbergii* females diets containing around 60μg g^{-1} DW vitamin C and vitamin E 300μg g^{-1} DW are sufficient to ensure proper reproduction and offspring viability, and might increase larval quality. Habashy (2003b) noticed that *M. rosenbergii* fed diet supplemented with 3% vitamins had final growth rate greater than un-supplemented diet group.

1.2.5.6. Mineral requirement

Minerals are essential nutrient for the prawns and crustaceans. Shrimp can absorb some minerals such as calcium, magnesium, manganese and iron from rearing water (Kanazawa *et al*., 1984). Thus, Vijayan and Diwan (1996) suggested that the major source of calcium for Indian white prawn *Penaeus*

indicus, cuticular mineralization comes from the ambient water by direct absorption. The crustacean exoskeleton is extensively mineralized with calcium carbonate as the principal inorganic component, and small amounts of magnesium and phosphate salts (Passano, 1960). They have to mineralize the newly formed exoskeleton after molting and again demineralize the old exoskeleton in preparation for the next molt (Vijayan and Diawan, 1996).

Calcium is considered one of the essential minerals that serve and important to growth of crustacean. The main source for exoskeleton calcification in freshwater crustaceans is the stored calcium into their tissues such as gastrolith, midgut gland and haemolymph. Tissue calcium of the prawn may probably play a supporting role when there is a sudden post molt demand (Vijayan and Diwan, 1996). Potassium plays an important role in whole body ion regulation and osmoregulation in crustaceans (Pillard *et al*., 2002). Lack of potassium can result in hyperpolarisation of cell membrane potential and paralysis (Wang *et al*., 2002). Rath and Dube (1994) indicated that zinc (Zn) has a significant effect on growth promotion and survival rate in *M. rosenbergii* at range 50 - 90 mg, therefore Davis *et al.* (1993) recommended that zinc and copper are considered as essential nutrient for shrimp. Habashy (2003b) found that diet supplemented with 1% minerals for juvenile *M. rosenbergii* resulted better growth than other diets.

1.2.5.7. Protein energy ratio requirements

Freshwater prawn might have used the protein as an energy source due to the lower gross energy in the diet (Zaki, 1999). Nevertheless, carbohydrate can be used to spare protein, and it is the cheapest dietary energy for shrimp and prawn feeds (Klontz, 1980). However, it is necessary to maintain a proper balance among food ingredients, protein/starch ratio and energy/protein ratio (Bages and Sloane, 1981). Thus, a mixture of carbohydrate has been found

sometimes to be used for energy during starvation of various crustaceans (Clifford and Bricks, 1983). In addition, the required amount of the requirements for essential fatty acid is essentially supply as good source of energy to spare dietary protein requirements (Hebalah, 2008).

Despite, the fasting energy metabolism of prawn is dominated by carbohydrates, followed by lipids and proteins (Clifford and Bricks, 1983). However, use the best source of carbohydrate is critical point. Zaki (1999) used silos wheat by-product as carbohydrate source for fed *M. rosenbergii*, and obtained the poor growth and decreased energy content in prawn's bodies compared with other diet (yellow corn, wheat bran and unextracted rice bran).

1.2.6. Digestion trails

Generally, the feed digestibility is less in the early post larval stages of crustacean (Bengston *et al*., 1991). Bioavailability of feedstuffs is an important factor to consider, in part because it is related to the quantity of nitrogen absorbed by shrimp. Almost 78% of nitrogen from dietary protein excreted during high density cultivation, and released to the environment (Jackson *et al*., 2003). Low digestibility of feeds leads to accumulation of nitrogen wastes in water and soil, which in turn can lead to shrimp disease and higher death rates (Lin *et al*., 2006), as well as pollution and eutrophication of water. Digestibility of fishmeal varies with the freshness and type of the raw ingredients andprocessing during manufacture (Smith *et al*., 2000). There are different factors affect utilization of feedstuffs, such as the raw material, recipient species, whole fish or scraps, freshness, processing methods, and storage conditions of the meal (Terrazas-Fierro *et al*., 2010).

Prawns appear to be able to utilize complex carbohydrates better than simple ones such as glucose (New, 1990). Dietary monosaccharides are rapidly absorbed, but are poorly utilized. González-Peña *et al*. (2002) were studied the effects of dietary cellulose on digestion and absorption in *M.*

rosenbergii. They found that increasing a-cellulose reduced the apparent digestibility of both dry matter and protein, but cellulose itself is digestible.

The amino acid digestive utilization coefficients is one of the most important factors in preparing adequate shrimp feeds and there is an increasing interest in defining feedstuff quality using as criterion the coefficients of amino acid digestibility (Yang *et al*., 2009). Richard *et al*. (2011) estimated apparent digestibility coefficient (ADC) of dry matter, protein, and amino acids of juvenile black tiger shrimp, *Penaeus monodon* following fishmeal replacement by different levels of plant protein (corn gluten meal, wheat gluten, and rapeseed meal), they found that, digestibility of dry matter, protein and energy was also significantly lower in all fishmeal-replaced diets. In particular, leucine digestibility decreased by 26% at 100% replacement. These results are surprising as corn gluten meal is usually found to be a highly digestible protein source in teleosts such as tilapia (Wu *et al*., 1995). Dry matter, energy, crude protein, gross energy and amino acid apparent digestibility coefficients (ADCs) in white shrimp juveniles *L. vannamei* were determined by Cruz-Suárez *et al*. (2009) for four soybean products, Terrazas-Fierro *et al*. (2010) for some foodstuffs, Nieto-López *et al*. (2011) for six wheat products, and Oujifard *et al*. (2012) forrice protein.

1.2.7. Histological studies

1.2.7.1. Hepatopancreas

The hepatopancreas, known as the midgut gland or digestive gland, has similar anatomical and histological structure as in all crustaceans but is different in the shape. The anatomy of hepatopacreas for some species of crustaceans described by Aly (2000), Sousa and Petriella (2000; 2006) and Calvo *et al*. (2011). They noticed that, it is dorsally located and surround the midgut.

Many reports have been studied the types of hepatopancreas cells of several species of crustaceans. Smith *et al*. (1975) observed two cell types hepatopancreas from *Acellus intermedius* (freshwater isopod), large alpha cells and small beta cells. Ying (1988) divided of hepatpancreas cells of *Penaeus orientalis* into three types based on the function. As well, Schultz (2005) studied the hepatopancreas of the freshwater amphipod (*Gummarus minus*), and he noticed that hepatopancreatic epithelium of Amphipoda more closely approximates that of Decapoda than that of the Isopoda. Aly (2000) observed that, hepatpancreas of crayfish *Procambrous clarkii* composed of numerous tubules. These tubules consist of two main types of cells; while Sayed (2002) described four cell types of hepatopancreatic tubules in the same species. Corrêa *et al*. (2002) was investigated hepatopancreatic tissue of the *Ucides cordatus* (crab), and noticed four epithelial cell types lined of hepatopancreatic tubules. Also, Mandal *et al*. (2003) found that, hepatopancreas of *Homarus americanus* (Lobster) is heterogeneous tissue composed of four epithelial cell types. In addition, Calvo *et al*. (2011) noticed that, the structure of the hepatopancreas of red claw crayfish *Cherax quadricarinatus* resembles that of other decapod crustaceans, thus observed four types of cell lining. Sousa and Petriella (2000) and Sousa *et al*. (2005) observed four cell types in the hepatopancreas of the freshwater prawn *Palaemonetes argentines*. Also, Bray *et al.* (2006) and Li *et al*. (2008) described four types of cells of hepatopancreas of marine shrimp *Litopenaeus vannamei*. Sagi and Ra'anan (1988) found relationship between weight of hepatopancreas and testis of *M. rosenbergii* during morphotypic differentiation. Also, Vázquez Boucard *et al*. (2004) observed depletion of nutritional reserves in hepatopancreas before spawning of *Fenneropenaeus indicus* female (shrimp).

During the molting cycle, histological changes of cell type composition of hepatopancreas have been observed for most crustaceans, such as *Palaemonetes argentines* (Sousa and Petriella, 2001), *Marsupenaeus japonicus* (Zilli *et al*., 2003), *Porcellio scaber* (Leser *et al*., 2008) and *Cherax quadricarinatus* (Calvo *et al*., 2011). Fernández Gimenez *et al*. (2008) observed the effect of different levels of vitamin A on the hepatopancreatic cells morphology of red shrimp *Pleoticus muelleri.* Also, Li *et al*. (2008) noticed disorders of the number and size of hepatopancreas cell types of white shrimp *Litopenaeus vannamei* at three ambient salinities. Aly (2000) studied effect of two types of insecticide on histology of hepatopancreas of *Procambrus clarkii* female (crayfish) from Nile River and he indicated reduced the size and increased number of hepatopancreaic cells. Thus, Archanachai (2005) indicated to the sensitivity of hepatopancreas to endosulfan (insecticide) for *Marcobracium lanchesteri* and he noted many tissue lesions of hepatopancreas. Bray *et al*. (2006) added Oxytetracyclin (antibiotic) to *Litopenaeus vannamei* feeds and noticed a reduction of lipid droplet storage in hepatopancreas.

1.2.7.1. Gonads

The reproductive system in *M. rosenbergii* consists of a pair of gonads and a pair of genital pores, which lie between the dorsal surface of the hepatopancreas and the heart (Sreekumar *et al*., 1982); the detailed anatomy of *M. rosenbergii* testis was carried out by Poljaroen *et al*. (2010).

Lee and Chang (1997) divided the ovarian development of *M. rosenbergii* to five developmental stages (Stages I–V), and they were measure the concentrations of vitellogenin and protein in hemolymph, ovary, and hepatopancreas. Meeratana and Sobhon (2007) classified the differentiating oocytes during ovarian cycle of *M. rosenbergii* into four different phases based on amount of lipid droplets and yolk granules. Likewise, Ferreira *et al*. (2012)

described five ovarian stages of *M. amazonicum* during the ovarian development.

Many studies have been by several authors to report the histology of *M. rosenbergii* testis. Lynn and Clark (1983) described the histological structure of sperm in details. Sagi *et al*. (1988) were studied spermatogenesis and sperm storage of three morphotypes. Poljaroen *et al*. (2010) were classified the developing male germ cells into 12 stages. Meeratana *et al*. (2006) indicated the effects of serotonin on ovarian development in *M. rosenbergii*; they observed that serotonin induced ovarian development and oocytes maturation. Sarojini *et al*. (1986) investigated drastic changes in the larvae and gonads of *M. lamerrii* by effect of fenitrothion (insecticide). As well as, Aly (2000) noted caused deformation of oocytes of *Procambrus clarkii* due to two types of insecticides. Whereas, Bray *et al.* (2006) added Oxytetracyclin (antibacterial) to diets of *Litopenaeus vannamei*, but no lesions were observed in gonad tissue.

1.3. Acidifiers

Both the feed industry and the food production sector suffer from losses due to the contamination of feed with pathogenic bacteria and their related impacts in the animal, such as lower weight gains or increased mortality. Therefore, public opinion and regulation authorities in most export countries focus now on the misuse of antibiotics (AGPs) in aquaculture and public attention has shifted towards production methods (Verbeeke, 2001; Feedinfo, 2005). The uses of antibiotics in-feed lead to: imbalance of the gut micro-flora, reduced efficacy of antibiotic therapeutics, the development of resistant bacteria strains, increased spread of salmonella and clostridium and risk of epidemic diseases, and possibility to transfer bacterial immunity to species pathogenic in animals and humans (Liem, 2007; Budiati *et al*., 2013). Therefore, the European Union has banned all antibiotics growth promoters

from livestock production this occurred in January 2006, since the use of low levels of these antibiotics in animals' feeds (Liem, 2004). Alterative feed ingredients are being adopted in order to fill the gap from the antibiotics. Thus, Acidifiers can be part of the feeding concept to replace it and it is expected that the market segment for this feed additive will continue to rise (Desai *et al*., 2007).

Acidifiers consist of organic acids and salts, they have been used for more than 30 years to reduce bacterial growth and mould in feedstuffs and thus preserve hygienic quality such as propoinic acid (Freitag, 2007). In animal feed, acidifiers were initially used in piglets to complement their limited capacity to maintain a low gastric pH, which is linked to problems with digestion (Easter, 1988). Also, acidifiers are more selective in their activity- they can reduce harmful micro-organisms and promote beneficial microflora colonization of the gastrointestinal tract (Mathew *et al*., 1991).

1.3.1. Organic acids

1.3.1.1. Characteristics

The use of organic acid salts or blends is an interesting option to promote the growth performance and health of a wide variety of aquaculture species worldwide (Lückstädt, 2006a) and terrestrial animals (Goosen *et al*., 2011). In addition, they are aiding in pathogen inhibition in the intestinal tract, physiological balance, providing energy to aquatic organisms, and improving the digestibility of dietary nutrients, such as nitrogen and phosphorus (Da Silva *et al*., 2013).

The effect of organic acids in the reduction of pH and their antimicrobial activity vary considerably depending on their dissociation status. The amount of dissociation depends on the pH value of the environment, and is described by the specific pK (dissociation constant) value for each acid, which defines the pH at a 50% dissociation rate. The lower pK

value, the stronger the acid, which relates to its ability to lower pH of the environment. Acids used as feed additives have pK values between 3 and 5, and are categorized as being of intermediate strength (Table 2). The nutritive value differs considerably between acids, and is highest in propionic acid and lowest in formic acid and its salts. For handling considerations, solid acids are easy to use, while liquid forms tend to be corrosive (Freitag, 2007).

1.3.1.2. Role in feed hygiene

Acidifiers are improving the performance and the health of the livestock if they are added to feed in sufficient amounts. Also, acids used as feed additives are predominantly compounds that naturally occur in cell metabolism, thus they are natural products with low toxicity. In animal nutrition, acidifiers exert their effects on performance via three different mechanisms in the feed, the gastro-intestinal tract of the animal, and effects on the animal's metabolism. Health and performance promoting effects have been demonstrated for a number of organic acids, including formic, fumaric, citric and lactic acid and their salts (Kirchgessner and Roth, 1988; Freitag, 2007).

A certain level of contamination with fungi, bacteria or yeasts is unavoidable in nutrient-rich products like feeds. Under favorable conditions microbes multiply rapidly during storage, especially at higher moisture levels in warm environments. Acidifiers function as conserving agents by reducing the pH the feed, thereby inhibiting microbial growth and thus lowering the uptake of possibly pathogenic organisms and their toxic metabolites (Freitag, 2007). Malicki *et al*. (2004) found that a mixture of formic and propionic acid (1% dosage) can act synergistically against *Escherichia coli* in stored fishmeal, which is an often-used ingredient in aqua feeds.

1.3.1.3. Role in metabolism

Most organic acids have high gross energy values (Table 2). Specially, short-chain organic acids are absorbed through the intestinal epithelia by passive diffusion and they can be used in various metabolic pathways for energy generation, for instance, fumaric acid is used as acidifier in human nutrition and the chemical industry, and fumaric salt is generated during the decomposition of aspartate, phenylalanine and tyrosine amino acids, in the ornithine cycle and during purine synthesis in animal metabolism. It is metabolically generated as part of the citric acid cycle, and involved in ATP generation in the cell (Freitag, 2007).

As the energy content of organic acids is completely used in metabolism it should be included in the energy content of feed rations. For example, propionic acid contains up to five times more energy than wheat (Diebold and Eidelsburger, 2006).

1.3.1.4. Role in digestive system

The mode of action of organic acids in the intestinal tract involves two different mechanisms: reduce the pH level in the stomach, particularly in the small intestine, through delivery of H^+ ions, and inhibit growth of gram-negative bacteria through the dissociation of the acids and the production of anions inside bacterial cell (Lückstädt, 2008).

During periods of high feed intake, such as when the animals are young or when the feeds are high in protein, hydrochloric acid concentrations in the stomach are reduced (Table 3). This reduction negatively impacts pepsin activation and pancreatic enzyme secretion and impairs digestion. Providing acidifiers in the feed addresses this problem and aids feed digestion (Eidelsburger, 1997). Positive effects of organic acids on protein hydrolysis have been demonstrated (Mroz *et al*., 2000). Similarly, feed supplementation

Table 2. Chemical properties of selected acids and salts.

Organic acid/salt	pK value	Solubility in water	Gross energy(KJ/kg)	Physical condition
Formic acid	3.75	Very good	5.8	liquid
Acetic acid	4.75	Very good	14.8	liquid
Propionic acid	4.75	Very good	20.8	liquid
Lactic acid	3.08	good	15.1	liquid
Fumaric acid	3.03/4.44	low	11.5	solid
Citric acid	3.14/5.95/6.39	good	10.3	solid
Na-formate	-	Very good	3.9	solid
Calcium lactate	-	low	10.2	solid
Calcium propionate	-	good	16.6	solid

(Kirchgessner and Roth, 1991)

with organic acid has been shown to lead to lower duodenal pH, improved nitrogen retention and increased nutrient digestibility (Øverland *et al*., 2000; Kluge *et al*., 2004). Whereas, the gastric acidity influences the bioavailability of dietary minerals (Wood and Serfatty-Lacrosniere, 1992) by regulating the chelation and complex formation of the element and by altering the transport mechanisms of minerals (Ravindran and Kornegay, 1993). On the other hand, the dietary acidification by citric acid increased whole body minerals. Therefore, the higher absorption of many minerals in diets supplemented with organic acid such as citric acid can increase the apparent digestibility, especially calcium, phosphorus and iron in rainbow trout, *Oncorhynchus mykiss* (Vielma and Lall 1997; Sugiura *et al*., 1998; Vielma *et al*., 1999; Pandey and Satoh, 2008) and phosphorus in Nile tilapia, *O. niloticus* (Goda *et*

al., 2002) and sea bream, *Pagrus major* (Sarker *et al*., 2005; Hossain *et al*., 2007).

Table 3. Effects of organic acids and their salts in animal nutrition.

	Effective form	**Effects**
Feed	H^+	-pH reduction -Reduction of acid binding capacity
	H^+ and Anion	-Reduction of microbial growth -Antibacterial effects
Intestinal tract	H^+	-pH reduction in stomach and duodenum -Improved pepsin activity
	Anion	-Complexing agents for cations (Ca^{++}, Mg^{++}, Fe^{++}, Cu^{++}, Zn^{++}) -Antibacterial effects
	$H^+$and Anion	-Change in microbial concentrations
Metabolism		-Energy supply

(Kirchgessner and Roth, 1988)

Gislason *et al*., (1996) noticed that the gut contents of Arctic charr, *Salvelinus alpines* fed a diet supplemented with sodium lactate contained less water, energy, lipid, protein and free amino acids; and did not diarrhea occur, probably indicating much lower amounts of residual nutrients and water in the gut. It was also proposed that the growth-promoting effect of dietary lactate in Arctic charris the result of the relatively slow gastric emptying rate.

1.3.1.5. Role in disease resistances

Acidifiers stimulated that the impact of bacterial infections can be reduced, and leading to higher survival rates (Lückstädt, 2006b). The growth rates of many gram-negative bacteria, such as *E. coli* or *salmonella spp,* are reduced below pH 5. Low pH also forms a natural barrier against microbes ascending from the ileum and large intestine. Moreover, low molecular-weight acids are lipophilic and can diffuse across the cell membranes of gram-negative bacteria. In the more alkaline cytoplasm, they dissociate and reduce the pH. This reduction alters cell metabolism and enzyme activity, thus inhibiting the growth of intralumenal microbes, especially that of pathogens. Several studies have demonstrated a reduction in bacterial counts in the stomach (Kluge *et al*., 2004) and the duodenum (Hebeler *et al*., 2000), while acid tolerant, beneficial *Lactobacilli* seem to be unaffected or may even be enhanced in number (Hellweg *et al*., 2006). Likewise, Potassium diformate (KDF) is stronger antimicrobial effect towards coliform bacteria than toward *Lactobacilli* (Février *et al*., 2001), leading to a more favorable microbiota with lower population levels of *E.coli* and *Salmonella* and higher population level and diversity of Lactobacilli (Hebeler *et al*., 2000).

Ringø (1991) observed that lactate prevented diarrhea in Arctic charr, *Salvelinus alpines*, while no such symptoms were observed in Atlantic salmon, *Salmo salar* (Gislason *et al*., 1994). It is generally accepted that short organic acids influence intestinal microbiota of animals (Lee *et al*., 2007; Lević *et al*., 2007; Owens *et al*., 2008). Iba and Berchieri (1995) tested a mixture of formic and propionic acid at a dosage of 2 kg/t against different salmonella serotypes. Vázquez *et al*. (2005) observed that lactic acid bacteria culture is only effective if supply the turbot *Scophthalmus maximus* host with organic acids.

1.3.2. Acidifiers in Aquaculture

Since the use of fish silage from preserved fish and fish viscera included the acid preservation (Åsgård and Austreng, 1981) in-feed acidifier came into scientific observation too (Lückstädt, 2006b). When, inorganic acid (sulphuric and hydrochloric acids) are used for preservation of fish silage; the pH of the silage has to be lowered to ≤ 2. Therefore, before feeding of silage to animals, the pH must be neutralized. On other hand, if organic acid such as formic or propionic acids are used, the silage is stable at pH levels of 3.5-4.0, enabling direct feeding without neutralization (Balios, 2003). Thus, in aquaculture, 2.2% formic acid was used to produce sardine fish hydrolysates for start feeding of sea bass *Dicentrarchus labrax* larvae (Kotzamanis *et al*., 2007).

Early study on the use of organic acids in fish diet included succonic and citric acids in diet for salmonids (Fauconneau, 1988). But the use of organic acids is not only tested in Salmoniformes, but also in other species. Ringø (1991, 1992) and Ringø *et al*. (1994) reported that addition of sodium lactate and acetate as an additive diets increased growth rate of Arctic charr, *Salvelinus alpines*. Similar study by Gislason *et al*. (1994, 1996) gave good result with Arctic charr, but no influence on growth of Atlantic salmon *Salmo salar*. Whereas, Christiansen and Lückstädt (2008) added potassium diformate to Atlantic salmon diet and noticed improved protein and fat digestibility.

In a recent trial, the inclusion of organic acid salts in fish diets was tested in rainbow trout, *Oncorhynchus mykiss*. De Wet (2005a, b) observed that the organic acid blend (formic acid and its salt plus ascorbic acid) improved the growth of rainbow trout better than Flavomycin. Also, Morken *et al*. (2011) were to evaluated the effects of sodium diformate on apparent nutrient digestibility and physical quality of diets of rainbow trout (*Oncorhynchus mykiss*); and Gao *et al*. (2011) were conducted to evaluate the

effects of adding an organic acid salt blend (mixture of sodium formate and butyrate) to diets for rainbow trout.

Owen *et al.* (2006) noted that no effects of sodium butyrate as a feed additive on the growth of catfish *Clarias gariepinus.* While, Hossain *et al*. (2007) showed good weight gain of red sea bream *Pagrus major* fed diet supplemented with various organic acids. In addition, Qi *et al*. (2012) investigated the effects of dietary taurine (2-aminoethanesulfonic acid) on feeding, growth, feed utilization of turbot juvenile, *Scophthalmus maximus*, and found that 0.5% taurine in diet of turbot with 165.9±5.01 g weight are probably optimal.

Many trials used organic acids and salts as feed additives to diets of Nile tilapia, *O. niloticus*. Abdelhamid *et al*. (1998) studied the effects of graded levels of gibberellic acid in diets differing in the crude protein levels on performance and chemical composition of Nile Tilapia fingerlings, and found that the improving effects were due to the lower gibberellic acid concentrations than the higher one. Xie *et al*. (2003) evaluated the effects of several organic acids (citric, metacectonic, lactic, acetic, and oxalic) on the stimulatory feeding behavior of Nile tilapia. Kim *et al*. (2003) indicated that sufficient supplementation of dietary ascorbic acid or α-tocopheryl acetate had positive effects on growth performance of fingerling Nile tilapia. Bakr and Haggag (2005) studied the effects of supplementing a basal diet of *O. niloticus* with either 0.08 or 0.16 % sodium butyrate on growth performance, body composition, histological changes of the intestinal epithelium, nutrients digestibility, and immune responses and indicated that 0.08 % sodium butyrate stimulates the development and growth of the large and small intestine. Ramli *et al.* (2005) showed that potassium diformate (KDF) can be growth promoter and efficient tool to control bacterial infections in tilapia; they observed that the growth performance improved at 0.2 and 0.5% formate addition, while

survival rates of fish after the challenge with *Vibrio anguillarum* on days 10–30 were also significantly higher than the negative control. El-Husseiny *et al*. (2007) investigated the effect of dietary sodium chloride on Nile tilapia performance, and they found the growth performance improved with addition of sodium chloride to fish diet. Petkam *et al*. (2008) determined the effects of an acid salts blend (calcium formate, calcium propionate, calcium lactate, calcium phosphate and citric acid) at different levels on the growth performance of *O. niloticus*, and noticed that the blend at 1.5% resulted increased weight gain. Zhou *et al*. (2009) investigated the effect of potassium diformate and two widely-used antibiotics, flavomycin and quinocetone, on growth performance, feed conversion ratio and gut microbiota of hybrid tilapia (*O. niloticus*♀× *O. aureus*♂), the results indicated that the addition of dietary potassium diformate and antibiotics had no significant effect on tilapia growth performance, but they effected on the gut microbiota.

Researches about the use organic acid as feed additives are somewhat limited in non-fish aquaculture species. Tung *et al.* (2006) mentioned that sodium citrate (5%) boosted the growth of the Kuruma shrimp, *Masurpenaeus japonicus*. Aftabuddin *et al*. (2009) used chemicals and biological products used in shrimp *P. monodon* hatcheries and grow-out ponds in Bangladesh. Nhan *et al*. (2010) investigated the effect of poly β-hydroxybutyrate (PHB) on the culture performance of larvae of the giant freshwater prawn *M. rosenbergii* and on the bacterial levels inside the larval gut, found that the PHB addition result the best overall culture performance since it significantly improved larval survival as well as larval development. Da Silva *et al*. (2013) selected salts of organic acid such as sodium acetate, sodium butyrate, sodium citrate, sodium formate, sodium lactate and sodium propionate, with potential to be used as feed additives for *L. vannamei*. On the other hand, Baruah *et al*. (2005, 2007) investigated the synergistic effects of citric acid and phytase on bone

mineralization and nutrient digestibility and growth performance in Indian carp, *Labeo rohita* juveniles.

The recent data include the successful usage of acidifiers in the development of artificial diets for abalone *Haliotis midae* (molluscea) in South Africa. Goosen *et al*. (2011) investigated organic acids (acetic acid, formic acid, benzoic acid, and sorbic acid) and organic acid blend (sodium benzoate and potassium sorbate) as growth promoters in cultured South African abalone when incorporated as feed additives in formulated feed.

2. MATERIALS AND METHODS

This chapter was conducted at El-Kanater El-Khayria Fish Research Station, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt during the period from June to December 2009. Three experiments were carried out to investigate the effect of dietary sodium lactate, calcium lactate and calcium propionate levels as organic acids salts on growth, nutrient digestibility, proximate body composition, feed utilization and histological induces of Nile tilapia, *Oreochromis niloticus* and freshwater prawn, *Macrobrachium rosenbergii*.

2.1. Aquatic animals

2.1.1. Nile tilapia

Nile tilapia, *O. niloticus* fingerlings with an initial body weight of 10.98± 2.63 g were obtained from Saft-Khalid hatchery, General Authority for Fish Resources Development, El-Behira Hatchery, El-Behira Governorate, Egypt. Prior to the start of experiment, nine hundred fingerlings were stocked in cement ponds (42 m^3). During this period, fish were fed a commercial diet (40%) at 3% of body weight. The daily ration was divided into three equal amounts and offered three times a day (08.00, 12.00 and 14.00 h). The cement ponds were supplied with freshwater from the Darawa Irrigation Branch, Kalubiya Governorate. The fish were held under natural light (12:12 h light: dark schedule).

2.1.2. Freshwater prawn

Freshwater prawn, *Macrobrachium rosenbergii* post-larvae (PL) an average initial body weight 1.54 ±1.11g and 0.30±0.18g for experiment I and II, respectively. The prawn was bought from Fish Hatchery, Mariut Company, Alexandria Governorate, Egypt. Prior to the start of experiment, eight

thousand juveniles were stocked in 6 fiberglass tanks (each $1m^3$) for acclimated to the experimental conditions for two weeks. During this period, prawns were fed a commercial diet (40%) at 5% of body weight. The daily ration was divided into three equal amounts and offered three times a day (08.00, 12.00, and 14.00 h). The tanks were supplied with freshwater from the Darawa Irrigation Branch, Kalubiya Governorate. The prawns were held under natural light (12:12 h light: dark schedule).

2.2. Culture techniques

2.2.1. Experiment II (Nile tilapia)

The experiment was conducted from 1st August 2009 to 2ed December 2009 (124 days). Nine hundred Nile tilapia, *O. niloticus* fingerlings were stocked into four cement ponds. Each cement pond was divided into five equal compartments (net pens) by netting (each of 8 m^3) and each pen was stocked with 40 fish. Duplicate pens were randomly assigned to each treatment. All ponds were supplied with freshwater from the Darawa Irrigation Branch, Kalubiya Governorate where the water turnover rate was 0.3 m^3 pond twice weekly. During the experimental period, photoperiod was held under natural light (12:12 h light: dark schedule). Fish were fed at 3% of body weight daily. The daily ration was divided into two equal amounts and offered two times a day (09.00 and 14.00 h), 7 days a week.

2.2.1.1. Experimental diets

Ten isonitrogenous 38% crude protein (CP) and isocaloric 20 MJ/kg gross energy (GE) experimental diets were formulated. The control diet (Diet1) had no organic acid salts added. Diets 2, 3 and 4 each was supplemented with sodium lactate at levels of 2, 3 and 4 g kg/ diet, respectively, while diets 5, 6 and 7 each contained calcium lactate at levels of 2, 3 and 4 g kg/ diet, respectively. Diets 8, 9 and 10 each was supplemented

with calcium propionate at levels of 2, 3 and 4 g kg/ diet, respectively. The proximate chemical composition of the basal experimental diet is presented in Table (4).

2.2.2. Experiment II and III (Freshwater prawn)

2.2.2.1. Experiment II

The experiment was conducted from 18th June 2009 to 30th August 2009 (75 days). Eight hundred freshwater prawns, *M. rosenbergii* PL were divided into thirty groups and stocked into thirty cement pens (each 0.75 m^3) at Fish Research Station, El-Kanater El-Khayria, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt. Each pen was stocked with 60 prawns. Duplicate pens were randomly assigned to each treatment. All pens were supplied with freshwater from the Darawa Irrigation Branch, Kalubiya Governorate where the water turnover rate was 0.25 m^3 pen twice weekly. During the experimental period, photoperiod was held under natural light (12:12 h light: dark schedule). Prawns were fed at 5% of body weight daily. The daily ration was divided into two equal amounts and offered two times a day (09.00 and 14.00 h), 7 days a week.

Mortality and injured bodies due to cannibalism were recorded daily. Each pen was provided with 10-25cm long 16-20 mm diameter of black polyvinyl chloride (PVC) pipe to minimize the cannibalism during the molting as suggested by Mariappan and Balasundaram (2004).

2.2.2.1.1. Experimental diets

Ten isonitrogenous 38% crude protein (CP) and isocaloric 20 MJ/kg gross energy (GE) experimental diets were formulated. The control diet (Diet1) had no organic acid salts added. Diets 2, 3 and 4 each was supplemented with sodium lactate at levels of 2, 3 and 4 g kg/ diet, respectively, while diets 5, 6 and 7 each contained calcium lactate at levels of

2, 3 and 4 g kg/ diet, respectively. Diets 8, 9 and 10 each was supplemented with calcium propionate at levels of 2, 3 and 4 g kg/ diet, respectively. The proximate chemical composition of the basal experimental diets is presented in Table (4).

2.2.2.2. Experiment III

This experiment was conducted to clearly the results obtained from experiment II were the higher dietary level of sodium lactate caused higher mortalities. So, this experiment was designed to eluted the effect of using lower dietary levels of sodium lactate (1 and 1.5 g/kg diet) compared to the higher dietary levels (2, 3 and 4g/kg diet) and control group to obtained the optimum dietary levels of sodium lactate. This experiment was conducted from 22th August 2009 to 2^{ed} December 2009 (102 days). One thousand and eight hundred freshwater prawns, *M. rosenbergii* PL were divided into eighteen groups and stocked into eighteen cement pens (each 0.96 m^3). Each pen was stocked with 100 prawns. Triplicate pens were randomly assigned to each treatment. All pens were supplied with freshwater from the Darawa Irrigation Branch, Kalubiya Governorate where the water turnover rate was 0.25 m^3 pen twice weekly. During the experimental period, photoperiod was held under natural light (12:12 h light: dark schedule). The feeding rate begins from 20% and decreasing to 7 and 5%, according to FAO (2002). The daily ration was divided into two equal amounts and offered two times a day (09.00 and 14.00 h), 7 days a week.

Each pen was provided with 10-25cm long 16-20 mm diameter of black polyvinyl chloride (PVC) pipe to minimize the cannibalism during the molting as suggested by Mariappan and Balasundaram (2004). Mortality and injured bodies due to cannibalism were recorded daily.

Table 4. Ingredients and proximate composition (%) of the experimental basal diet used in the experiment I and II.

Ingredients(g|kg)	**Experimental Basal Diet***
Fish meal	340
Soybean meal	270
Wheat bran	250
Yellow corn	60
Soybean oil	20
Linseedoil	20
Vit. and minerals[1]	40
Proximate composition (% on dry matter basis)	
Dry matter	93.33
Crude protein	37.91
Crude fat	12.45
Total carbohydrate	37.89
GE (KJ /kg) [2]	20.46
P/E ratio (mg cp/KJ GE/g)[3]	18.07

*Diet1 (control diet without organic acid salts as acidifiers); diets 2, 3 and 4were containing 2, 3 and 4% sodium lactate; diets 5, 6 and 7 were containing 2, 3 and 4% calcium lactate and diets 8, 9 and 10 were containing 2, 3 and 4% calcium propionate.

[1]Vitamins and minerals premix per 1 kg: 4.8m. I.U, Vit A.,0.8m.I.U, VitD_3., 0.4g. Vit E., 0.8g. Vit K., 4.0g. VitB_{12}., 4.0g. VitB_2., 0.6g.Vit B_6., 4.0g. vit Pantothenic acid., 8.0g. vit Nicotinic acid., 400mg vit Folic acid ., 20mg. vit Biotin., 200g. Choline., 4g. Copper.,0.4g. Iodine., 12g. Iron., 22g. Manganese., 22g. Zinc.,0.04g. Selenium.

[2]GE: Gross energy, calculated using gross caloric values of 5.65, 9.45 and 4.2 kJ/g for protein, fat and carbohydrate, respectively according to Hepher *et al* (1983).

[3]P/E ratio: protein energy ratio.

2.2.2.2.1. Experimental diets

Six isonitrogenous 38% crude protein (CP) and isocaloric 20 MJ/kg gross energy (GE) experimental diets were formulated. The control diet

(Diet1) without organic acid salts added. Diets 2-6 each was supplemented with sodium lactate at levels of 1, 1.5, 2, 3 and 4 g kg/ diet, respectively. The proximate chemical composition of the experimental basal diet is presented in Table (5).

2.3. Preparation of experimental diet

The diets were processed by blending the dry ingredients into a homogenous mixture. Pellets of two mm were made with a home mixture grinder (PHILIPS, Mode HL 1616ID, Philips India Limited. 7, Justice Chandra Medhab Road, Calcutta 700020). All diets were dried (temperature did not exceed 40ºC) until the moisture was 10%, then packed in cellophane bags and stored at -4ºC prior to use. Dietary gross energy (GE) contents were calculated according to gross caloric values of Hepher *et al* (1983) using the values of 5.65, 9.45 and 4.2 kcal/g for crude protein, crude fat, and total carbohydrate, respectively.

Table 5. Ingredients and proximate composition (%) of the experimental basal tested diet used in the experiment III.

Ingredients(g|kg)	**Experimental Basal Diet***
Fish meal	340
Soybean meal	270
Wheat bran	250
Yellow corn	60
Soybean oil	20
Linseedoil	20
Vit. and minerals[1]	40
Proximate composition (% on dry matter basis)	
Dry matter	93.33
Crude protein	37.91
Crude fat	12.45
Total carbohydrate	37.89

GE (KJ /kg) [2]	20.46
P/E ratio (mg cp/KJ GE/g)[3]	18.07

*Diet 1 (control diet without organic acid salts as acidifiers); diets 2, 3, 4, 5 and 6 were containing 1, 1.5, 2, 3 and 4% sodium lactate.

[1]Vitamins and minerals premix per 1 kg: 4.8m. I.U, Vit A., 0.8m.I.U, VitD_3., 0.4g. Vit E., 0.8g. Vit K., 4.0g. VitB_{12}., 4.0g. VitB_2., 0.6g.Vit B_6., 4.0g. vit Pantothenic acid., 8.0g. vit Nicotinic acid., 400mg vit Folic acid ., 20mg. vit Biotin., 200g. Choline., 4g. Copper.,0.4g. Iodine., 12g. Iron., 22g. Manganese., 22g. Zinc.,0.04g. Selenium.

[2]GE: Gross energy, calculated using gross caloric values of 5.65, 9.45 and 4.2 kJ/g for protein, fat and carbohydrate, respectively according to Hepher *et al* (1983).

[3]P/E ratio: protein energy ratio.

2.4. Growth performance and Feed utilization

To estimate the growth performance indices during the experimental period, initial and final as well as intermediate samples weights of individual Nile tilapia fingerlings and freshwater prawn PL were measured using an electronic balance (Demer instrument APX-60).

2.4.1. Weight Gain (WG)

WG was calculated by the following equation:

WG= final body weight (FBW) g– initial bodyweight (IBW) g

Where: FBW= Weight at the end of period, IBW= Weight at zero time.

2.4.2. Specific Growth Rate (SGR)

The SGR was calculated by the following equation:

SGR = ln FBW – ln IBW x 100 / Rearing period (days).

Where: ln= natural logarithmic

2.4.3. Survival (S %)

The S was calculated by the equation:

SR = (final number of samples / initial number of samples) ×100.

2.4.4. Feed Conversion Ratio (FCR)

The FCR was determined as follows:

FCR = feed intake (g)/weight gain (g)

2.4.5. Protein Efficiency Ratio (PER)

PER was determined as follows:

PER = weight gain (g) /Protein intake.

Where: Protein intake = Food consumed × Percent of protein in diet.

2.4.6. Protein Productive Value (PPV)

PPV was determined of protein at the end of the rearing period for fish and prawn in each pond as follows:

PPV= Protein gain / Protein intake.

Where: Protein gain = Final Body Protein – initial Body Protein

2.4.7. Fat ratio (FR)

FR was determined of fat at the end of the rearing period for fish and prawn in each pond as follows:

FR= Fat gain / Fat intake.

Where: Fat gain = Final Body Fat – initial Body Fat

2.4.8. Energy Utilization (EU)

EU was determined as follows: EU = energy gain (kJ)/energy intake (kJ).

2.5. Proximate Composition

At the beginning and end of the trial, random three pooled groups samples of 5 healthy individual of *M. rosenbergii* and *O. niloticus*, respectively were selected, weighed and immediately sacrificed and frozen at -20 ºC for assessment of the initial and final proximate body composition for each experiment.

The chemical composition of fish and diet samples were determined according to the procedures of AOAC (1995). Dry matter was determined after drying the samples in an oven (105°C) for 24 h. Ash by incineration at 550°C for 12 h. Crude protein was determined by micro-Kjeldhal method, %N × 6.25 (using Kjeltechautoanalyzer, Model 1030, Tecator, Höganäs, Sweden) and crude fat by Soxhlet extraction with diethyl ether (40 – 60°C).

2.6. Digestibility trial

The apparent digestibility coefficient (ADC) of different experimental diets was determined as described by procedure of Nwanna (2003) using the sedimentation technique for last experimental two weeks. A total 150 of Nile tilapia with an average weight of 28g were randomly stocked into 30 glass aquaria (70 × 41 × 55cm) with a capacity of 45 L of water. The glass aquaria were filled with well water source. Water was continuously aerated to supply oxygen. After two weeks of fish (or prawns) acclimation the experimental fish (or prawns) were stocked as 50 animals /aquaria.

Ten experimental diets (Table 6) were formulated to content chromic oxide (Cr_2O_3) as an external marker at a level of 0.05% of the diet. Half an hour after feeding, un-eaten feed was collected from each aquaria, then feces was collected. The water in the aquariums was siphoned off three times a day, always after each feeding, and at the end of the day. About 20–50% of the water volume of the aquarium was replaced, which was sufficient to remove

nutrients dissolved in water and faecal waste remaining materials. Faeces proximate analysis was followed the method described by the AOAC (1995). Chromic oxide concentration was determined according to the method described by Edwards and Gillis (1959). Dry matter, protein, energy, and minerals apparent digestibility coefficients (ADCs) of diets were calculated using the following equations as described by Maynard *et al*. (1981):

$$ADC_{\text{dietary nutrient}} = 1 - ((\text{marker}_{\text{diet}}) / (\text{marker}_{\text{feces}}) \times (\text{nutrient}_{\text{feces}}) / (\text{nutrient}_{\text{diet}})).$$

Chromic oxide was determined by the method of Edwards and Gills (1959) using spectrophotometer after dried at 80ºC for 24 hours and digesting with nitric acid and perchloric acid.

2.7. Statistical analysis

Data were statistically analyzed by ANOVA using MSTATE-C version 4 software (MSTATE-C 1987). The assay data were submitted to Bartlett test to verify homoscedasticity. The data showed no variances in homogeneity. Subsequently, the data were submitted to one ways classification variance analysis. Duncan's multiple range test was used to compare differences between treatment means when significant F values were observed (Duncan, 1955), at ($P \leq 0.05$) level. All percentage data were arc-sin transformed prior to analysis (Zar, 1984), however data are presented untransformed to facilitate comparisons.

2.8. Histological works

Randomly 4 specimens from each replicate were dissected. The organs (liver and gonads from tilapia, hepatopancreas and gonads from prawn) were transferred to the fixing fluid. Bouin's fluid is used as a general fixative and gave satisfactory results. The material was dehydrated, cleared and finally

embedded in paraffin wax. Serial sections were cut to the thickness of 5-6µ. The sections were stained with heamatoxylin counter stained with eosin and mounted in DPX (Humason, 1979). The sections were examined by Olympus light microscope and photographed with digital camera as required. The histological examination was carried out at Zoology Department, Faculty of Science, Ain Shams University.

Table 6. Ingredients and proximate composition (%) of experimental basal diet used for digestibility trial in the experiment I and III.

Ingredients(g|kg)	**Experimental Basal Diet***
Fish meal	340
Soybean meal	270
Wheat bran	250
Yellow corn	60
Soybean oil	20
Linseedoil	20
Vit. and minerals[1]	40
Chromic oxide	0.5
Proximate composition (% on dry matter basis)	
Dry matter	93.33
Crude protein	37.91
Crude fat	12.45
Total carbohydrate	37.89
GE (KJ /kg) [2]	20.46
P/E ratio (mg cp/KJ GE/g)[3]	18.07

*Diet 1 (control diet without organic acid salts as acidifiers); diets 2, 3 and 4 were containing 2, 3 and 4% sodium lactate; diets 5, 6 and 7 were containing 2,3 and 4% calcium lactate and diets 8, 9 and 10 were containing 2, 3 and 4% calcium propionate.

[1]Vitamins and minerals premix per 1 kg: 4.8m. I.U, Vit A., 0.8m.I.U, VitD_3., 0.4g. Vit E., 0.8g. Vit K., 4.0g. VitB_{12}., 4.0g. VitB_2., 0.6g.Vit B_6., 4.0g. vit Pantothenic acid., 8.0g. vit Nicotinic acid., 400mg vit Folic acid ., 20mg. vit Biotin., 200g. Choline., 4g. Copper.,0.4g. Iodine., 12g. Iron., 22g. Manganese., 22g. Zinc.,0.04g. Selenium.

[2]GE: Gross energy, calculated using gross caloric values of 5.65, 9.45 and 4.2 kJ/g for protein, fat and carbohydrate, respectively according to Hepher *et al* (1983).

[3]P/E ratio: protein energy ratio.

3. RESULTS AND DISCUSSION

3.1. Growth rates

3.1.1. Experiment I (Nile tilapia)

All conditions of the experimental evaluation were apparently satisfactory, and fell under the optimal standards defined for nutritional evaluations in Nile tilapia, *O. niloticus* fingerlings. Proximate composition of the experimental diet is shown in Table (4). The basal experimental diet was formulated to contented 37.91% CP, 12.45 % crude fat, 37.89% total carbohydrate, 20.46 kJ/kg gross energy (GE) and 18.07mg CP KJ/GE/g protein energy ratio (P: E ratio) according to Hepher *et al* (1983).

Average initial body weight of *O. niloticus* fingerlings was 10.98± 2.63 g. No significant difference in initial weight among the experimental treatments was observed, indicating the accuracy of randomization process between and within the experimental treatments.

Table (7) showed the growth performance including final body weight (FBW), weight gain (WG), specific growth rate (SGR) and survival (S %) of Nile tilapia, *O. niloticus* fingerlings at different organic acid salts as acidifiers (sodium lactate, calcium lactate and calcium propionate) with different levels (0, 2, 3 and 4%) compared to control (without organic acid salts). At different organic acid salts as acidifiers, no significant differences ($P \geq 0.05$) of FBW, WG, SGR and survival (S %) was recorded among different organic acid salts as acidifiers. The highest values ($P \geq 0.05$) of FBW, WG, and SGR were observed for fish fed diet contain sodium lactate (147.5g, 122.2g and 2.120%/day respectively), while the lowest values were recorded with diet supplemented with calcium lactate (136.4g, 111.2g and 2.023%/day respectively). The highest value of S% was noted with control group (93.75%), and the lowest one was recorded with sodium lactate (86.25%).

At different organic acid salts levels (Table 7), no significant difference ($P \geq 0.05$) was observed for S%. The S% highest value was recorded with control group (93.75%), and the lowest value was recorded at 3% level (86.19%). The highest significant values ($P \leq 0.05$) of FBW, WG, and SGR were observed with 4% acidifiers levels (157.5g, 132.3g and 2.20 %/day respectively), while, the lowest significant values ($P \leq 0.05$) was recorded with 3% organic acid salts levels (132.6g, 107.3g, 1.99 %/day, respectively).

Ringø *et al*. (1994) and Gislason *et al.* (1996) had not observed any effect of dietary sodium lactate on Atlantic salmon juveniles, *salmo salar* L. compared to Arctic charr, *Salvelinus alpines* L. and Nile tilapia, except for slower enhanced in specific growth rate SGR. Likewise, Zhou *et al*. (2009) had no recorded any effect for dietary potassium diformate (KDF) supplementation on hybrid tilapia (*Oreochromis niloticus* ♀×*O. aureus* ♂) growth performance or survival compared to the control group. Ringø *et al*. (1994) found slightly increased Survival rates in Atlantic salmon juveniles feeding on sodium lactate. The same result was recorded in the present work.

Tung and Pettigrew (2006) reported that, diet acidification significantly reduces the diet pH but does not affect the gastrointestinal pH**.** Acidifiers in the diet act as performance promoters by lowering the pH of gut mainly upper intestinal tract (Desai *et al*., 2007) and a larger inhibition effect against possible pathogenic bacteria (Sissons, 1989). The effect of lactic acid is probably related to environmental pH. Up to its pK value of 3.9, lactic acid is mainly present in its non-polar un-dissociated form due to lower polarity, in such case; it has anti-bacterial activity only in the fish stomach when pH is reach 3.0, while intestinal pH ranged between 8.0 and 9.0 in small and large intestine (Gislason *et al.,* 1996; Levital *et al*., 2009). Therefore, acidification

of diet significantly reduces the diet pH but does not affect the gastrointestinal pH.

In this book, the levels of dietary organic acid salts as acidifiers of 4% improved growth performance of Nile tilapia compared with other levels, this conflict with Xie *et al*. (2003), they found that different dietary lactic acid (10^{-5} to10^{-2} M) had stimulatory effects on the Nile tilapia feeding response and /or biting rate and without any significant differences among the four levels ($P>0.05$).

At the interaction among different organic acid salts as acidifier levels, the best growth performance observed with fish fed on sodium lactate at a level of 4% compared with other experimental treatments (Table 9). In contrast, the highest final weight of Arctic charr (Ringø, 1991) was obtained with fed on diet with 1% sodium lactate. Bakr and Haggag (2005) of Nile tilapia and Owen *et al.* (2006) of catfish, *Clarias gariepinus* found that improving effects of growth performance and chemical composition at lower dietary levels of sodium butyrate, than the higher.

In present work, the highest significant value of survival was recorded with the fish fed on 4% calcium lactate, while the lowest value ($P\leq0.05$) was observed with 3% sodium lactate this result may due to its greatest antifungal activity of calcium lactate than sodium lactate (Moon, 1983). Aran (2001) and Stonerock (2007) reported that calcium lactate was inhibitory than sodium lactate as the growth of bacterial spores and may be related with the efficacy of organic acid against some microbes like salmonella depends on their pK value and molecular size of organic acid, whatever, the salts of lactic acid are a more powerful pathogen inhibitor than the lactic acid itself. And it is important for protection tilapia from some bacteria, specially, transfer of antibiotic resistance genes to other bacteria in *Tilapia mossambica* (Budiati *et al*., 2013).

Table (8) illustrated the feed utilization of Nile tilapia, *O. niloticus* fed different organic acid salts as acidifiers sources and levels. No significant differences ($P \geq 0.05$) were recorded for feed conversion ratio (FCR), protein efficiency ratio (PER), protein productive value (PPV), fat ratio (FR), and energy utilization (EU). The best FCR value was recorded with calcium propionate (1.48), and the highest worth value was showed with control group (1.68). The lowest values of PER, PPV, FR and EU (1.733, 25.41%, 38.27% and 22.10%, respectively) were recorded with sodium lactate. While, the highest values of PER and PPV were recorded for group of fish fed diet supplemented with calcium propionate (2.006 and 27.65%, respectively). With respect to FR and EU the highest values were observed with calcium lactate (40.79 and 22.27%, respectively).

Irrespective of organic acid salts as acidifiers sources (Table 8), no significant differences ($P \geq 0.05$) were observed for FCR, PPV and EU at different organic acid salts levels (0, 2, 3 and 4%). The best FCR value was recorded at 4% organic acid salts levels (1.43), and the highest worth value (1.68) was noted with control group (0%).

PER and FR showed a significant difference ($P \leq 0.05$) among different organic acid salts as acidifiers levels (Table 8). Likewise, the lowest values of PPV and EU were observed with 2% organic acid salts levels (24.52 and 20.86% respectively), while the highest values was recorded with 4% (30.58 and 24.05%, respectively).

Growth performance, Survival (%) and feed utilization of Nile tilapia, *O. niloticus* fed different organic acid salts as acidifiers sources and levels and their interaction effects is presented in Table (9). Significant differences ($P \leq 0.05$) were observed for of all parameters of growth performances at different levels of organic acid salts. No significant differences ($P \geq 0.05$) were

observed for feed utilization among different experimental treatment except for FR.

The highest significant values ($P \leq 0.05$) of FBW, WG and SGR were recorded for fingerlings fed on diet content 4% of sodium lactate (162.2g, 136.9g, and 2.23%/day, respectively); meanwhile the lowest significant values were observed at 2% of calcium lactate (122.7g, 97.45g, and 1.90%/day, respectively). No significant differences ($P \leq 0.05$) were observed for FCR, PER, PPV and EU the interaction effects between the different organic acid salts as acidifier sources and levels. In addition, fish fed diet supplemented calcium propionate at a level 4% recorded the lowest value (32.61%) of FR, while the highest (51.84%) was observed for fish fed diet supplemented with 3% calcium propionate (Table 9).

De Wet (2005b) noticed that Feed conversion ratio tended to be lower with increasing levels of the acid blend (formate and sorbate), even if compared to flavomycin antibiotic group. Ramli *et al.* (2005) found that 2% of the potassium diformate lead to an improvement in weight gain and FCR in tilapia. In this connection Sarker *et al*. (2005) reported on red sea bream, *Pagrus major*, they observed that the improving growth performance and FCR was with 3% citric acid, whereas 1% in other study (Hossain *et al*., 2007) due to reduce the use of inorganic phosphours. Recently, Gao *et al*. (2011) found that diets supplemented with a sodium formate and butyrate blend did not improve growth rate or feed utilization of rainbow trout, *Oncorhynchus mykiss*.

Table 7. Growth performance and Survival rate (%) of Nile tilapia, *O. niloticus* fed on different organic acid salts as acidifiers sources and levels.

	FBW	WG	SGR	S (%)
Acidifiers sources				
Control (0%)	140.8±0.08^{a}	115.5±0.08^{a}	2.070±0.00^{a}	93.75±3.75^{a}
Na-lactate	147.5±1.05^{a}	122.2±1.23^{a}	2.120±0.13^{a}	86.25±1.86^{a}
Ca-lactate	136.4±2.40^{a}	111.2±2.61^{a}	2.023±0.18^{a}	93.13±4.87^{a}
Ca-propionate	142.9±2.02^{a}	117.7±2.31^{a}	2.077±0.19^{a}	89.02±7.03^{a}
Acidifiers levels				
0%	140.8±0.08^{b}	115.5±0.08^{b}	2.070±0.00^{ab}	93.75±3.75^{a}
2%	138.3±2.26^{b}	113.0±2.48^{b}	2.031±0.19^{b}	89.17±6.47 ^{a}
3%	132.6±1.36^{b}	107.3±1.62^{b}	1.993±0.11^{b}	86.19±4.11 ^{a}
4%	157.5±1.94^{a}	132.3±1.28^{a}	2.199±0.14^{a}	88.75±6.73 ^{a}

Means values (±) in the same column sharing the same subscript are not significantly different (P≥ 0.05).
FBW = Final body weight (gm), WG= Weight gain (gm), SGR= Specific growth rate (%), S (%) =Survival rate.

Table 8. Feed utilization of Nile tilapia, *O. niloticus* fed on different organic acid salts as acidifier sources and levels.

	FCR	PER	PPV	FR	EU
Acidifier sources					
Control (0%)	1.68±0.44[a]	1.73±0.46[a]	26.21±6.77[a]	38.27±12.47[a]	21.41±5.95[a]
Na-lactate	1.61±0.26[a]	1.74±0.29[a]	25.41±3.52[a]	40.00±4.61[a]	22.10±2.52[a]
Ca-lactate	1.66±0.45[a]	1.81±0.59[a]	26.87± 1.22[a]	40.79±6.88[a]	22.27±6.35[a]
Ca-propionate	1.48±0.21[a]	2.01± 0.37[a]	27.65±4.84[a]	40.34±10.12[a]	21.25±3.18[a]
Acidifier levels					
0%	1.68±0.44[a]	1.73±0.46[ab]	26.21±6.77[a]	38.27±12.47[b]	21.41±5.95[a]
2%	1.64±0.39[a]	1.70±0.33[b]	24.52±4.13[a]	38.07±3.91[b]	20.86±3.44[a]
3%	1.57±0.25[a]	1.80±0.32[ab]	25.26±3.10[a]	45.87±6.48[a]	21.18±2.47[a]
4%	1.43±0.33[a]	2.17±0.54[a]	30.58±7.58[a]	39.30±8.65[b]	24.05±5.75[a]

Means values (±) in the same column sharing the same subscript are not significantly different (P≥ 0.05).
FCR= Feed conversion ratio, PER=Protein efficiency ratio, PPV=Protein productive value, FR=Fat retention, EU=energy utilization.

Table 9. The interaction effects of different dietary organic acid salts as acidifiers sources and levels on growth performance, survival (%) and feed utilization of *O. niloticus* (Wet weight basis).

Experimental treatments										
Sources		**Na- lactate**			**Ca- lactate**			**Ca- propionate**		
Levels	**Control (0%)**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**
FBW	140.8±0.1^{abc}	147.3±6.0^{abc}	139.6±3.1^{abc}	162.2±2.3^{a}	122.7±5.2^{c}	124.7±1.4^{c}	157.6±8.8^{ab}	144.7±3.6^{abc}	133.5±1.0^{bc}	152.8±2.4^{ab}
WG	115.5±0.1^{abc}	122.1±6.0^{abc}	114.4±3.1^{abc}	136.9±2.8^{a}	97.45±5.7^{c}	99.40±1.8^{bc}	132.4±9.3^{a}	119.5±3.1^{abc}	108.3±1.5^{abc}	127.5±2.8^{ab}
SGR	2.07±0.1^{abc}	2.12± 0.1^{abc}	2.06±0.1^{abc}	2.23±0.2^{a}	1.90±0.1^{c}	1.92±0.1^{bc}	2.20±0.2^{a}	2.07±0.3^{abc}	2.00±0.1^{abc}	2.17±0.1^{ab}
S (%)	93.75±3.8^{ab}	91.25±8.8^{ab}	77.50±2.0^{b}	82.50±5.0^{ab}	90.00±7.5^{ab}	93.75±6.3^{ab}	95.00±0.1^{a}	86.25±3.8^{ab}	87.33±12.5^{ab}	88.75±6.2^{ab}
FCR	1.68±0.4^{a}	1.63±0.3^{a}	1.69±0.4^{a}	1.45±0.1^{a}	1.88±0.6^{a}	1.62±0.2^{a}	1.44±0.5^{a}	1.42±0.1^{a}	1.41±0.2^{a}	1.40±0.4^{a}
PER	1.73±0.5^{a}	1.64±0.3^{a}	1.61±0.3^{a}	1.96±0.1^{a}	1.56±0.5^{a}	1.69±0.2^{a}	2.27±0.8^{a}	1.91±0.1^{a}	2.10±0.2^{a}	2.28±0.6^{a}
PPV	26.21±6.8^{a}	23.94±3.9^{a}	23.72±4.11^{a}	27.75±1.4^{a}	22.55±6.1^{a}	25.48±2.3^{a}	33.24±1.7^{a}	27.07±0.6^{a}	26.58±3.2^{a}	30.74±8.3^{a}
FR	38.27±2.4^{ab}	38.22±5.6^{ab}	43.11±5.9^{ab}	40.39±0.2^{ab}	37.35±5.3^{ab}	42.66±3.3^{ab}	44.89±1.3^{ab}	38.65±0.8^{ab}	51.84±6.4^{a}	32.61±8.8^{b}
EU	21.41±6.0^{a}	21.57±3.5^{a}	22.02±3.2^{a}	23.42±0.1^{a}	19.08±5.3^{a}	21.42±2.5^{a}	27.18±8.7^{a}	21.93±0.3^{a}	20.10±2.2^{a}	21.54±5.7^{a}

Means values (±) in the same raw sharing the same subscript are not significantly different (P≥ 0.05).

FBW = Final body weight (gm), WG= Weight gain (gm), SGR= Specific growth rate (%), S (%) =Survival rate, FCR= Feed conversion ratio, PER=Protein efficiency ratio, PPV=Protein productive value, FR=Fat ratio, EU=energy utilization.

Proximate body composition of *O. niloticus* fingerlings fed different organic acid salts as acidifiers sources and levels compared to control diet is shown in Table (10). Irrespective of organic acid salts levels, the highest significant value ($P \leq 0.05$) of moisture (MO) was obtained with fish group fed calcium propionate (73.36%) and the lowest value was recorded for control fish group (72.18%). The opposite trend was observed for whole body content of crude protein (CP), Ether extract (EE), Ash, and gross energy.

Irrespective of organic acid salts as acidifier sources (Table 10); results showed that CP, EE and Ash had the higher significant values ($P \leq 0.05$) for fish fed control diet (14.09, 6.63 and 4.47%, respectively). No clear trend was observed as the interaction effects of different dietary organic acid salts as acidifier sources and levels on proximate body composition of *O. niloticus* (Table 11).

Aran (2001) observed that calcium lactate at a level of 3% reduced the pH values from 6.0 to 5.5 and it was inhibitory than sodium lactate as the growth spores of *Bacillus cereus*. With respect to EE and GE, the highest values ($P \leq 0.05$) observed with fish fed on 3% sodium lactate, this may related to the complex relationship between metabolism and organic acids, whereas the energy content of organic acids is made completely available during metabolism (Diebold and Eidelsburger, 2006). Also, the inhibition of pathogenic intestinal micro-organisms helps to absorbed along the digestive tract, providing an additional energy source for animal (Roth *et al*., 1993).

Table 10. Proximate body composition of *O. niloticus* fed on different organic acid salts as acidifier sources and levels (Wet weight basis).

	MO	CP	EE	ASH	GE*
Acidifier sources					
Control (0%)	72.18±0.18 [b]	14.09±0.57 [a]	6.63±0.57 [a]	4.47±0.28 [a]	594.94±0.04 [a]
Na-lactate	72.19±0.52[b]	13.76±0.98[a]	6.79±0.81[a]	4.18±0.32[a]	593.47±0.09[a]
Ca-lactate	72.29±1.68[b]	13.96±1.29[a]	6.49±0.99[a]	4.16±0.49[a]	586.34±0.07[a]
Ca-propionate	73.36±1.02[a]	13.22±1.01[b]	5.70±1.07[b]	3.79±1.07[b]	537.63±0.14[b]
Acidifier levels					
0%	72.18±0.18[c]	14.09±0.57[a]	6.63±0.57[a]	4.47±0.28[a]	594.94±0.04[a]
2%	73.11±1.29[a]	13.51±0.89[bc]	6.08±0.83[b]	4.17±0.34[b]	559.50±0.07[a]
3%	72.43±1.05[bc]	13.75±1.45[ab]	6.40±1.17[ab]	3.64±0.99[c]	577.82±0.17[a]
4%	72.74±1.63[ab]	13.24±1.06[c]	6.19±1.35[b]	3.88±0.65[c]	557.47±0.14[a]

Means values (±) in the same column sharing the same subscript are not significantly different (P≥ 0.05).
MO: Moisture, CP: Crude protein, EE: Ether extract, GE: Gross energy
*Gross energy (GE) calculated using gross caloric values of 23.63, 39.52 and 17.56 kJ/g for protein, Ether extract and carbohydrate, respectively according to Hepher *et al* (1983).

Table 11. The interaction effects of different dietary organic acid salts as acidifier sources and levels on proximate body composition of *O. niloticus* (Wet weight basis).

Experimental treatments										
Sources		**Na- lactate**			**Ca- lactate**			**Ca- propionate**		
Levels	**Control (0%)**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**
MO (%)	72.18±0.2^{d}	72.36±0.4^{cd}	72.11±0.8^{d}	72.13±0.3^{d}	73.25±2.0^{b}	72.18±1.1^{d}	71.56±1.5^{d}	73.73±0.4^{b}	72.99±1.1^{bc}	74.53±0.8^{a}
CP (%)	14.09±0.6^{b}	13.73±1.0^{bc}	13.82±0.8^{bc}	13.40±1.2^{bc}	13.55±1.0^{bc}	14.36±2.0^{a}	13.85±0.8^{bc}	13.26±0.8^{c}	13.06±1.3^{cd}	12.47±0.7^{d}
EE (%)	6.63±0.6^{abcd}	6.43±0.8$^{bcd e}$	7.27±1.0^{a}	6.81±0.2^{abc}	6.05±1.1^{de}	6.18±0.4^{cde}	7.11±1.0^{ab}	5.76±0.3^{e}	5.74±1.3^{e}	4.66±1.0^{f}
Ash (%)	4.47±0.3^{a}	4.29±0.4^{ab}	4.03±0.2^{bc}	3.93±0.2^{bc}	4.23±0.3^{ab}	3.84±0.6^{bc}	4.08±0.5^{bc}	3.99±0.2^{bc}	3.05±1.5^{d}	3.64±1.0^{c}
GE(kJ/g)*	262.0±0.0^{c}	254.1±0.1^{d}	287.3±0.1^{a}	269.1±0.0^{c}	239.1±0.1^{d}	244.2±0.1^{d}	281.0±0.0^{b}	227.6±0.0^{d}	226.8±0.2^{d}	184.2±0.1^{e}

Means values (±) in the same raw sharing the same subscript are not significantly different (P≥ 0.05).MO= Moisture. CP= Crude protein. EE= Ether extract

*Gross energy (GE) calculated using gross caloric values of 23.63, 39.52and 17.56kJ/g for protein, Ether extract and carbohydrate, respectively according to Hepher *et al* (1983).

Table (12) showed the apparent protein digestibility (APD) and apparent lipid digestibility (ALD) coefficients of different organic acid salts as acidifiers (sodium lactate, calcium lactate and calcium propionate), different levels (0, 2, 3 and 4%) at different organic acid salts as acidifiers' diets by *O. niloticus*. The lowest significant value ($P \leq 0.05$) of APD and ALD were observed with control group (73.32 and 49.46%, respectively), while, the highest significant values ($P \leq 0.05$) of APD was recorded with calcium propionate (82.95%) and for ALD with calcium lactate group (68.44%).

In respect to different levels (Table 12), the results showed that, the lowest significant values ($P \leq 0.05$) of APD and ALD were recorded for control group (0%), as well, the highest significant values ($P \leq 0.05$) of APD for fish fed on 2% (86.22%), and for ALD for those fed on 3% which represented by 64.96%.

Table (13) showed interaction effects of different dietary organic acid salts as acidifiers sources and levels on the apparent protein digestibility (APD) and apparent lipid digestibility (ALD) coefficients of experimental diets for *O. niloticus*. The highest significant value ($P \leq 0.05$) of APD (87.98%) was observed at 2% calcium lactate, while the lowest one ($P \leq 0.05$) was for group of fish fed on 3% of the same organic acid salt and represented by 72.59.

With respect to ALD, the highest significant value ($P \leq 0.05$) was found for those fed on 4% sodium lactate which represented by 81.77, while the value decreased to the lowest value (49.46%) for fish fed on control diet (0%).

In the present book, at different organic acid salts as acidifier sources, the apparent protein digestibility (APD) and lipid digestibility (ALD) were improved with fish fed on calcium propionate and calcium lactate, respectively. The previous results agree with those obtained by Kirchgessner

and Roth (1982) by using calcium propionate. Hossain *et al*. (2007) reported that the improved digestibility by organic acid is considered to be caused by; lowered pH resulting in a higher dissociation of mineral compounds, reduced rate of gastric emptying and formation of chelated mineral complexes, which are easily absorbed. Hence, the organic acids may improve the absorption of mineral such as Ca, P, Mg and Zn in the small intestine (Kirchgessner and Roth 1988). Thus, Partenen and Moroz (1999) demonstrated that organic acids improved the apparent digestibility protein and amino acids, which might influence mucosal morphology, as well as stimulate pancreatic secretions, because they might contribute to improved protein, energy and/ or mineral absorption. Therefore, Trinidad *et al*. (1999) suggested that the propionate enhanced the absorption of calcium in the intestine. Moreover, Ayyat *et al*. (2000) noticed the improvement in the apparent protein digestibility of *O. niloticus* related with copper absorption from intestine.

At different organic acid salts as acidifier level, the apparent lipid digestibility (ALD) of *O. niloticus* was increased with increased dietary levels, but the highest value of the apparent protein digestibility (APD) observed with 2%. These results were similar to report by Bakr and Haggag (2005), who found that the highest improvement in CP and EE digestibilities of *O. niloticus* were recorded with the basal diet supplemented with 0.08% sodium butyrate than higher level (0.16%) due to improved immune status.

Based on, the interaction among different organic acid salts as acidifier levels, the best result of the apparent protein digestibility (APD) was observed with diet containing 2% calcium lactate and the apparent lipid digestibility (ALD) with 4% sodium lactate. These results were similar to studies by Storebakken *et al*. (2010) reported an improvement in amino acid digestibility in Atlantic salmon (*Salmo salar*) when adding 12 g kg^{-1} potassium diformate (KDF) to diets; and Morken *et al*. (2011), who indicated that sodium diformate

(NaDF) at 10.6 g kg^{-1} concentration to diet of rainbow trout**,** *Oncorhynchus mykiss* increased the digestibility of most major nutrients and individual amino acids of diets. While Gao *et al*. (2011) found that the supplementation of OAB (10 g acid moiety kg^{-1} of a mixture of sodium formate and butyrate, ratio 2:1) diet of *Oncorhynchus mykiss* reduced the digestibility of organic matter, crude fat, and most amino acids.

Table 12. Apparent protein digestibility (APD) coefficients and apparent lipid digestibility (ALD) coefficients of different organic acid salts as acidifiers diets by *O. niloticus* (mean±SD)[A].

	APD (%)	**ALD (%)**
Acidifiers sources		
Control (0%)	73.32±5.37 [b]	49.46±9.65[b]
Na-lactate	82.42±8.93[a]	62.03±23.70[a]
Ca-lactate	78.18±7.41[a]	68.44±7.93[a]
Ca-propionate	82.95±8.44[a]	62.93±12.17[a]
Acidifiers levels	73.32±5.37[b]	49.46±9.65[b]
0%	86.22±6.82[a]	65.08±23.51[a]
2%	81.37±11.12[a]	64.96±9.58[a]
3%	83.81±7.06[a]	78.37±6.52[a]

Different letters in the same column display differences according to Duncan's multiple range test (P≤0.05).

[A]Values are the mean of three replicates (3X6 prawns per replicate).

Table 13. The interaction effects of different dietary organic acid salts as acidifiers sources and levels on apparent protein digestibility (APD) coefficients and apparent lipid digestibility (ALD) coefficients of experimental diets of *O. niloticus* fingerlings (mean±S.d)[A].

Experimental treatments										
Sources		**Na- lactate**			**Ca- lactate**			**Ca- propionate**		
Levels	**Control (0%)**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**
APD(%)	73.32±5.4^{ab}	84.14±10.6^{ab}	87.06±10.4^{ab}	85.14±9.6^{ab}	87.98±6.2^{a}	72.59±1.0^{b}	78.81±0.3^{ab}	86.55±4.8^{ab}	84.47±14.4^{ab}	87.48±6.8^{ab}
ALD(%)	49.46±9.7^{c}	51.72±35.8^{bc}	65.19±14.1^{abc}	81.77±9.4^{a}	80.69±7.0^{a}	66.21±0.7^{abc}	77.40±5.5^{ab}	62.83±15.5^{abc}	63.48±12.8^{abc}	75.94±5.0^{abc}

Different letters in the same raw display differences according to Duncan's multiple range test (P≤0.05).
[A]Values are the mean of three replicates (3X6 prawns per replicate).

3.1.2. Experiment II (Freshwater prawn)

Growth performance and Survival (%) of freshwater prawn, *M. rosenbergii* fed on different organic acid salts as acidifier sources and levels is shown in Table 14. Average of initial body weight of *M. rosenbergii* PL was 1.54±1.11 g. No significant difference in initial body weight of prawns.

The highest significant values ($P \leq 0.05$) of FBW, WG and SGR were observed for prawn fed diet supplemented with either calcium lactate (11.41g, 9.87g and 2.11%/day, respectively) or calcium propionate (11.21g, 9.67g and 2.08%/day, respectively), while the lowest values ($P \leq 0.05$) was recorded for prawn fed diet supplemented with sodium lactate (8.66g, 7.12g and 1.77%/day, respectively). In the present work, beneficial effects of dietary organic acid salts as acidifiers was recorded on growth performance (FBW, WG, and SGR) of *M. rosenbergii* compared with control. Petkam *et al*. (2008) and Yuk *et al*. (2008) reported that dietary calcium lactate at a level 2% enhanced immune and health status and improves disease resistance from some bacteria such as *E. coli*, thereby improving growth performance, in spite of the shrimps have no specific immune system, they have only innate immune system (Aftabuddin *et al*., 2009).

Irrespective of organic acid salts as acidifier levels, S (%) observed the highest values ($P \leq 0.05$) either control or calcium propionate groups (91.23 and 93.14%, respectively), while the lowest S (%) was recorded either sodium lactate or calcium lactate (84.56 and 84.99%, respectively). No significant ($P \leq 0.05$) effect of organic acid salts levels was observed on SGR and S (%) of freshwater prawn. In this connection, the highest significant values ($P \leq 0.05$) of FBW and WG were observed for fish fed diet organic acid salts level of 2% (11.63 and 10.09g, respectively). Da Silva *et al*. (2013) suggested that propionate had the biggest potential to be used in the diet supplementation of marine shrimp, *Litopenaeus vannamei* because it decreased the *Vibrio spp*

concentration in the intestinal tract, and the same effect noticed with probiotic (FLOC) on the same species (Aguilera-Rivera *et al*., 2014). Nhan *et al*. (2010) investigated the effect of poly β-hydroxybutyrate (PHB) on the culture performance of larvae of *M. rosenbergii* and on the bacterial levels inside the larval gut; they found that the feeding larvae with PHB-containing *Artemia nauplii* significantly improved survival and development of the larvae due to a growth-inhibitory effect towards these potentially pathogenic microorganisms, specially *Vibrio harveyi* (Pande *et al*., 2013).

Table (15) illustrated the feed utilization induces of freshwater prawn, *M. rosenbergii* fed on different organic acid salts as acidifier sources and levels. Prawn fed the diet supplemented with either sodium lactate or organic acid salts as acidifier levels at 3% had lower values of PER, PPV, FR, and EU and worth FCR compared to other experimental treatments. In general, prawns fed on either different organic acid salts as acidifier sources or levels recorded the highest feed utilization induces and best FCR values.

The interaction effects of different dietary organic acid salts as acidifier sources and levels on growth performance, survival (%) and feed utilization of freshwater prawn, *M. rosenbergii* is shown in Table (16). The results showed the lowest significant values ($P \leq 0.05$) of FBW, WG, SGR and S were observed with the diet supplemented with 4% sodium lactate (7.19g, 5.65g, 1.54%/day and 69.93%, respectively). While, the highest significant values ($P \leq 0.05$) of FBW, WG and SGR were noticed for groups of prawn fed the diet containing 2% calcium lactate (13.61g, 12.07g and 2.30%/day, respectively). The same trend was observed for PPV and FR when prawns fed diet supplemented with 4% sodium lactate.

The highest significant value ($P \leq 0.05$) of S was recorded with 3% calcium propionate (97.92%) and the lowest one was recorded at 4% sodium lactate (69.93%). As well as, the best value of FCR was observed for diet

supplemented with 4% calcium lactate (1.47%) and the worth values was observed at 4% sodium lactate (2.76%).

The highest significant values ($P \leq 0.05$) of PER was recorded with 4% calcium lactate (2.06%) and for EU with control group which represented by 18.60% (Table 16).

Dietary 4% sodium lactate decreased all growth performance, Survival rate and feed utilization; this may suggest the negative effects of sodium lactate on health fish status of *M. rosenbergii*, these result is agreement with the results of Gislason *et al*. (1996), on Atlantic salmon, *Salmo salar*. Compared results obtained with control diet (0%) with other experimental diets, the results indicated that the highest value of all feed utilization observed with control. The best results of FCR and PER were with *M. rosenbergii* fed on 4% calcium lactate this may due to increasing the utilization efficiency of food intake. As well, the highest value ($P \leq 0.05$) of PPV and FR observed with freshwater prawn fed on 4% calcium propionate may be due to increase the dietary utilization of CP and EE with the relatively gastrointestinal pH declines (4.88) as the effect of dietary calcium propionate as reported by Kung *et al*. (2003).

Table 14. Growth performance and Survival (%) of freshwater prawn, *M. rosenbergii* fed on different organic acid salts as acidifier sources and levels.

	FBW	WG	SGR	S (%)
Acidifiers sources				
Control (0%)	10.26±2.89^{ab}	8.72±1.86^{b}	2.01±0.37^{a}	91.23±6.81^{a}
Na-lactate	8.66±2.73^{b}	7.12±2.01^{b}	1.77±0.55^{b}	84.56±15.19^{b}
Ca-lactate	11.41±4.28^{a}	9.87±3.38^{a}	2.11±0.49^{a}	84.99±7.98^{b}
Ca-propionate	11.21± 4.14^{a}	9.67±3.23^{a}	2.08±0.56^{a}	93.14±5.82^{a}
Acidifiers levels				
0%	10.26±2.72^{ab}	8.72±1.86^{b}	2.01±0.37^{a}	91.23±6.81^{a}
2%	11.63 ±4.12^{a}	10.09±3.29^{a}	2.12±0.54^{a}	88.86±4.53^{a}
3%	10.07±3.98^{ab}	8.52±3.30^{b}	1.94±0.56^{a}	86.91±11.99^{a}
4%	9.75±4.08^{b}	8.21±3.27^{b}	1.88±0.59^{a}	83.24± 16.85^{a}

Means values (±) in the same column sharing the same subscript are not significantly different (P≥ 0.05).
FBW = Final body weight (gm), WG= Weight gain (gm), SGR= Specific growth rate (%), S (%) =Survival rate.

Table 15. Feed utilization of freshwater prawn, *M. rosenbergii* fed on different organic acid salts as acidifiers sources and levels.

	FCR	PER	PPV	FR	EU
Acidifiers sources					
Control (0%)	1.73±0.30^{b}	1.61±0.34^{a}	25.18±5.35^{a}	11.36±2.42^{a}	18.60±4.07^{a}
Na-lactate	2.20±0.82^{a}	1.32±0.30^{b}	19.02±4.80^{b}	7.34±3.40^{b}	13.21±1.00^{a}
Ca-lactate	1.80±0.61^{b}	1.65±0.57^{a}	22.09±7.90^{a}	10.32±2.90^{a}	14.52±1.10^{a}
Ca-propionate	1.90±0.72^{b}	1.63±0.54^{a}	24.42±9.10^{a}	11.31±4.10^{a}	14.88±1.70^{a}
Acidifiers levels					
0%	1.73±0.30^{b}	1.61±0.34^{a}	25.18±5.35^{a}	11.36±2.42^{a}	18.60±4.07^{a}
2%	1.97±0.63^{ab}	1.47±0.43^{a}	20.16±5.84^{bc}	9.32±2.53^{b}	13.06±1.04^{bc}
3%	2.05±0.57^{ab}	1.46±0.49^{a}	18.97±7.20^{c}	8.78±4.10^{b}	11.47±0.80^{c}
4%	2.13±0.99^{a}	1.60±0.63^{a}	23.06±10.00^{ab}	9.17±5.50^{b}	13.68±1.80^{b}

Means values (±) in the same column sharing the same subscript are not significantly different (P≥ 0.05).
FCR= Feed conversion ratio, PER=Protein efficiency ratio, PPV=Protein productive value, FR=Fat ratio, EU=energy utilization.

Table 16. The interaction effects of different dietary organic acid salts as acidifiers sources and levels on growth performance, Survival (%) and feed utilization of freshwater prawn, *M. rosenbergii*.

Experimental treatments										
Sources		**Na- lactate**			**Ca- lactate**			**Ca- propionate**		
Levels	**Control (0%)**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**
FBW	10.26±2.9^{ab}	9.93±2.9^{b}	7.27±2.0^{c}	7.19±2.6^{c}	13.61±5.0^{a}	9.82±3.1^{b}	11.94±4.1^{a}	11.35±3.8^{a}	13.11±4.3^{a}	10.13±4.2^{ab}
WG	8.72±1.9^{b}	8.38±2.0^{b}	5.72±1.1^{c}	5.65±1.6^{c}	12.07±4.0^{a}	8.28±2.0^{b}	10.40±3.0^{ab}	9.81±2.8^{ab}	11.56±3.3^{a}	8.58±3.2^{b}
SGR	2.01±0.4^{bc}	1.96±0.5^{bc}	1.59±0.5^{bc}	1.54±0.6^{c}	2.30±0.6^{a}	1.94±0.4^{bc}	2.17±0.4^{a}	2.10±0.6^{ab}	2.28±0.6^{a}	1.93±0.6^{bc}
S (%)	91.23±9.6^{ab}	92.87±5.2^{ab}	84.19±12.9^{bc}	69.93±23.2^{c}	87.39±3.8^{bc}	78.62±10.6^{bc}	82.72±6.5^{bc}	86.32±2.0^{bc}	97.92±1.3^{a}	97.08±1.3^{a}
FCR	1.73±0.3^{bc}	2.02±0.5^{bc}	2.30±0.5^{ab}	2.76±1.2^{a}	1.84±0.7^{bc}	2.16±0.5^{ab}	1.47±0.4^{c}	2.04±0.7^{bc}	1.69±0.6^{bc}	2.14±0.9^{abc}
PER	1.61±0.3^{bc}	1.35±0.3^{c}	1.18±0.2^{c}	1.14±0.3^{c}	1.61±0.5^{bc}	1.31±0.3^{c}	2.06±0.6^{a}	1.44± 0.4^{c}	1.90±0.5^{ab}	1.59±0.6^{bc}
PPV	25.18±5.4^{ab}	20.97±4.9^{bc}	15.96±2.8^{cd}	13.97±3.8^{d}	22.09±7.1^{abc}	14.45±3.2^{d}	26.63±0.6^{ab}	17.42±0.4^{cd}	26.49±0.5^{ab}	28.58±0.6^{a}
FR	11.36±2.4^{ab}	10.22±2.4^{ab}	4.55±0.7^{c}	3.22±0.8^{c}	9.19±2.8^{b}	9.25±2.1^{b}	11.48±3.3^{ab}	8.54±2.4^{b}	12.53±3.5^{a}	12.82±4.9^{a}
EU	18.60±4.1^{a}	14.27±3.3^{bc}	11.50±2.0^{cd}	8.47±2.3^{d}	13.87±4.4^{bc}	9.43±2.0^{d}	16.17±4.5^{ab}	11.04±3.0^{cd}	13.48±3.6^{bc}	16.38±6.1^{ab}

Means values (±) in the same raw sharing the same subscript are not significantly different (P≥ 0.05).

FBW = Final body weight (gm), WG= Weight gain (gm), SGR= Specific growth rate (%), S (%) =Survival rate, FCR= Feed conversion ratio, PER=Protein efficiency ratio, PPV=Protein productive value, FR=Fat ratio, EU=energy utilization.

Proximate composition of whole body of *M. rosenbergii* fed on different organic acid salts as acidifier sources and levels is shown in Table (17). At different organic acid salts, no significant difference was observed for ash prawns content. The highest significant value ($P \leq 0.05$) of MO was noticed for prawns fed on calcium lactate (75.40%) and the lowest was recorded for control group (71.56%). Group of prawns fed on control diet showed the highest significant differences ($P \leq 0.05$) for CP, EE and GE which represented by 15.66, 3.01% and 2.22 kJ/g, respectively. Likewise, the lowest significant values ($P \leq 0.05$) of EE and GE were recorded for prawns fed sodium lactate diet (2.01% and 2.00 kJ/g, respectively) and for CP with calcium lactate (13.68%).

The present results showed that the highest value ($P \leq 0.05$) of MO was noticed for prawns fed on 3% (75.59%) and the lowest for those fed on control diet represented by 71.56%. Results showed the highest significant values ($P \leq 0.05$) of CP, EE, ASH and GE for control group which represented by 15.66, 3.01, 5.33% and 2.22 kJ/g, respectively (Table 15). While the lowest significant values ($P \leq 0.05$) of CP, EE and GE were recorded for prawn fed on 3% of acidifiers (13.35, 1.85% and 1.94 kJ/g, respectively) and for ash at 4% which represented by 4.36%.

The interaction effect of different dietary organic acid salts as acidifier sources and levels on proximate body composition of *M. rosenbergii* is shown in Table (18). The results showed that the highest significant value ($P \leq 0.05$) of MO and were recorded for prawns fed diet supplemented with 3% calcium lactate (78.48%). The highest values ($P \leq 0.05$) of CP, EE and GE were observed for prawns fed diet supplemented with 4% calcium propionate (17.72 and 3.22%, respectively).

Table 17. Proximate composition of whole body of *M. rosenbergii* fed on different organic acid salts as acidifier sources and levels (Wet weight basis).

	MO	CP	EE	ASH	GE*
Acidifiers sources					
Control (0%)	71.56±0.55^{c}	15.66±0.13^{a}	3.01±0.67^{a}	5.33±0.45^{a}	488.97±0.11^{a}
Na-lactate	73.12±2.19^{b}	14.59±1.18^{a}	2.01±0.76^{b}	4.73±0.45^{a}	424.17±0.06^{b}
Ca-lactate	75.40±2.13^{a}	13.68±1.16^{b}	2.20±0.28^{ab}	4.55±0.40^{a}	410.18±0.04^{b}
Ca-propionate	73.74±4.15^{b}	15.04±2.49^{a}	2.57±0.88^{a}	4.66±0.69^{a}	456.94±0.07^{ab}
Acidifiers levels					
0%	71.56±0.55^{c}	15.66±0.13^{a}	3.01±0.67^{a}	5.33±0.45^{a}	488.97±0.11^{a}
2%	75.32±2.79^{ab}	14.06±1.50^{b}	1.96±0.70^{b}	4.45±0.67^{b}	409.67±0.06^{b}
3%	75.59±3.42^{a}	13.35±1.32^{c}	1.85±0.53^{b}	4.44±0.42^{b}	388.55±0.07^{b}
4%	73.86±3.27^{b}	14.69±2.41^{b}	2.22±0.94^{b}	4.36±0.48^{b}	434.83±0.07^{b}

Means values (±) in the same column sharing the same subscript are not significantly different (P≥ 0.05).
MO = Moisture, CP = Crude protein, EE = Ether extract, GE: Gross energy.
*Gross energy (GE) calculated using gross caloric values of 23.63, 39.52 and 17.56 kJ/g for protein, Ether extract and carbohydrate, respectively according to Hepher *et al* (1983).

Table 18. The interaction effects of different dietary organic acid salts as acidifiers sources and levels on proximate body composition of *M. rosenbergii* (Wet weight basis).

	Experimental treatments									
Sources		**Na- lactate**			**Ca- lactate**			**Ca- propionate**		
Levels	**Control (0%)**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**	**2%**	**3%**	**4%**
MO (%)	71.56±0.6^{cd}	72.65±0.3^{c}	72.39±2.8^{cd}	75.87±0.4^{ab}	75.41±1.9^{b}	78.48±2.6^{a}	76.14±0.4^{ab}	77.91±2.6^{ab}	75. 89± 2.1^{ab}	69.58±1.0^{d}
CP (%)	15.66±0.1^{b}	15.60±0.2^{b}	14.08±0.2^{c}	13.01±0.6^{cbe}	13.96±0.9^{c}	11.79±0.6^{e}	13.32±0.8^{cd}	12.61±1.2^{de}	14.17±1.1^{c}	17.72.±1.2^{a}
EE (%)	3.01±0.7^{b}	2.31±1.2^{bc}	1.43±0.1^{c}	1.29±0.1^{c}	1.63±0.1^{c}	2.01±0.2^{bc}	2.14±0.3^{bc}	1.93±0.5^{bc}	2.10±0.8^{bc}	3.22±0.8^{a}
Ash (%)	5.33±0.5^{a}	4.89±0.2^{ab}	4.76±0.1^{abc}	3.96±0.0^{d}	4.76±0.3^{abc}	3.92±0.0^{d}	4.19±0.9^{cd}	3.71±0.7^{d}	4.64±0.3^{bc}	4.95±0.3^{ab}
GE(kJ/g)*	119.0±0.1^{b}	91.3±0.1^{c}	56.5±0.1^{e}	51.0±0.1^{e}	64.4±0.1^{d}	79.4±0.1^{cd}	84.6±0.1^{cd}	76.3±0.1^{d}	83.0±0.1^{cd}	127.3±0.1^{a}

Means values (±) in the same raw sharing the same subscript are not significantly different (P≥ 0.05).
MO= Moisture. CP= Crude protein. EE= Ether extract, GE: Gross energy.
*Gross energy (GE) calculated using gross caloric values of 23.63, 39.52and 17.56kJ/g for protein, Ether extract and carbohydrate, respectively according to Hepher *et al* (1983).

3.1.3. Experiment III (Freshwater prawn)

The effect of different dietary sodium lactate (Na-lactate) levels Growth performance and feed utilization of freshwater prawn, *M. rosenbergii* PL is shown in Table 19. Average of initial body weight of *M. rosenbergii* PL was 0.30±0.18g. No significant difference either initial body weight or S% of prawn used in this experiment.

The results showed that, the highest significant values ($P \leq 0.05$) of FBW, WG, SGR, PER, PPV, FR and EU were observed for prawns group fed the diet supplemented with 1% Na-lactate (1.33g, 1.07g, 4.21%/day, 2.69, 34.75, 7.37 and 20.93%, respectively). The same trend was recorded for the best FCR. However, the lowest values ($P \leq 0.05$) of FBW, WG, SGR, PER and EU were recorded for prawn fed the diet supplemented with 2% Na-lactate which represented by 0.96g, 0.70g, 3.60, 1.53 and 14.88%, respectively. The lowest significant values ($P \leq 0.05$) of PPV and FR were recorded with dietary Na-lactate at 3% (26.81 and 3.52%, respectively).

The best significant values ($P \leq 0.05$) of FCR value was observed for prawns group fed the diet supplemented with 1% Na-lactate, while the worth values were observed for prawns control diet.

The studies of shrimp or prawn fed with organic acids or their salts are limited. The present results are in agreement with other authors. Lückstädt (2008) found that the addition of 0.5% sodium citrate with inactivated lactobacilli resulted in the highest growth of the shrimp *Marsurpenaeus japonicus*. As well, Kühlmann *et al*. (2011) showed an increase of 19% on productivity in comparison to non-supplemented and improves growth performance of white-leg shrimp, *Litopenaeus vannamei* shrimp fed on diet supplemented with 0.5% of potassium diformate. Also, Da Silva *et al*. (2013) noticed increased the growth and feed intake of *L. vannamei* fed on the lower

dietary levels of concentration sodium butyrate and sodium propionate (2%). But, Anuta *et al*. (2011) observed that the addition of 0.4–2% commercial acid, based on calcium sulfate, did not change the performance parameters of *L. vannamei*; however, they noted an increase in the immune response and a change in the intestinal microbiota.

Proximate body composition of freshwater prawn, *M. rosenbergii* fed different dietary Na-lactate levels are shown in Table (20). No significant difference was observed for body EE content. No clear trend was observed as the effects of different dietary Na-lactate levels on proximate body composition of prawn PL, *M. rosenbergii* except for the higher values recorded for body CP and GE for post larvae fed diet containing 2% Na-lactate.

ASH content showed the highest significant value ($P \leq 0.05$) for control group (5.77%) and lowest one (3.85%) for prawns fed on 1.5% sodium lactate.

The proximate composition of *M. rosenbergii* PL (Wet weight basis) and Survival (%) at different levels of sodium lactate showed no significant differences; generally, the highest value observed for prawns fed on 1.5% sodium lactate. The other study suggested that 0.25% calcium formate improved *Penaeus monodon* survival in Taiwan farms (Lückstädt, 2008). This may indicate that 1.5% sodium lactate has antimicrobial effects (Lückstädt and Mellor, 2011).

Table 19. Growth performance and feed utilization of freshwater prawn, *M. rosenbergii* fed different dietary sodium lactate (Na-lactate) levels.

Treatments	FBW	WG	SGR	FCR	PER	PPV	FR	EU	S (%)
Control (0%)	0.65±0.2^{d}	0.39±0.1^{d}	2.70±1.2^{c}	3.12±0.6^{a}	0.90±0.2^{d}	12.43±2.8^{c}	2.82±1.3^{d}	7.50±1.9^{c}	85.21±0.8^{a}
1%	1.33±0.7^{a}	1.07±0.6^{a}	4.21±1.4^{a}	1.71±1.3^{c}	2.69±1.6^{a}	34.75±20.9^{a}	7.37 ± 5.0^{a}	20.93±12.6^{a}	84.50±10.5^{a}
1.5%	1.09±0.6^{bc}	0.83±0.5^{bc}	3.75±0.9^{b}	1.85±0.9^{b}	2.30±1.3^{b}	27.90±16.4^{b}	5.50±3.6^{bc}	16.25±9.5^{b}	88.50±6.5 ^{a}
2%	0.96±0.4^{c}	0.70±0.2^{c}	3.60±1.0^{b}	1.84±0.5^{b}	1.53±0.5^{c}	22.86±8.0^{b}	4.47±1.8^{bcd}	14.88±5.2^{b}	78.50±17.5^{a}
3%	1.15±0.7^{bc}	0.89±0.5^{bc}	3.88±0.8^{b}	1.72±0.8^{c}	1.98±1.1^{bc}	26.81±15.5^{b}	3.52±4.5^{cd}	18.50±10.7^{ab}	72.00±17.0^{a}
4%	1.20±0.7^{b}	0.94±0.6^{b}	3.93±0.8^{b}	1.75±1.0^{b}	2.28±1.4^{b}	27.75±16.7^{b}	6.14±3.9^{ab}	16.84±10.2^{ab}	84.50±4.5^{a}

Means in the same column sharing the same subscript are not significantly different (P≥ 0.05).
FBW = Final body weight (gm), WG= Weight gain (gm), SGR= Specific growth rate (%), FCR= Feed conversion ratio, PER=Protein efficiency ratio, PPV=Protein productive value, FR=Fat retention, EU=energy utilization, S (%) =Survival rate

Table 20. Proximate body composition of freshwater prawn, *M. rosenbergii* fed different dietary sodium lactate (Na-lactate) levels (Wet weight basis).

Treatments	MO	CP	EE	ASH	GE*
Control (0%)	72.11±0.0^{b}	14.66±0.2^{ab}	1.61±0.4^{a}	5.77±0.1^{a}	410.02±0.1 ^{a}
1%	74.63±1.6^{b}	13.52±0.8^{b}	1.21±0.2^{a}	3.91±0.3^{c}	367.27±0.1^{b}
1.5%	76.63±0.9^{a}	13.04±0.4^{b}	1.29±0.2^{a}	3.85±0.1^{c}	359.09±0.1^{b}
2%	72.02±3.1^{b}	15.20±1.7^{a}	1.30±0.3^{a}	4.54±0.5^{b}	410.53±0.1 ^{a}
3%	72.39±2.8^{b}	14.09±1.5^{ab}	1.42±0.1^{a}	4.76±0.4^{b}	389.04±0.1^{b}
4%	75.87±0.4^{b}	13.00±0.4^{b}	1.30±0.1^{a}	3.96±0.1^{c}	358.54±0.1^{b}

Means values (±) in the same column sharing the same subscript are not significantly different (P≥ 0.05). MO = Moisture, CP = Crude protein, EE = Ether extract, GE: Gross energy.

*Gross energy (GE) calculated using gross caloric values of 23.63, 39.52 and 17.56 kJ/g for protein, Ether extract and carbohydrate, respectively according to Hepher *et al* (1983).

The apparent protein digestibility (APD) and apparent lipid digestibility (ALD) coefficients of different sodium lactate concentrations diets (1, 1.5, 2, 3 and 4%) and control (0%) of *M. rosenbergii* PL are shown in Table (21). The results indicated that, the highest significant values ($P \leq 0.05$) of APD and ALD were recorded for prawns fed on 4% sodium lactate (84.18 and 68.08%, respectively). Whereas, the lowest values ($P \leq 0.05$) of APD and ALD was for those fed on 1.5 sodium lactate and represented by 73.81% and 46.89%, respectively.

This may indicate that higher dietary Na-lactate was required to improve the digestibility of dietary nutrients, such as nitrogen and phosphorus. Similar results were obtained by Hossain *et al*. (2007) reported on red sea bream *Pagrus major*, who found the growth of fish fed on 1% lactic acid diet was significantly lowest among all diets but the apparent energy digestibility value was significantly higher than of 0.5% inorganic phosphorus diet. As well, Da Silva *et al*. (2013) found that sodium propionate increased the coefficient of apparent digestibility of energy and phosphorus in the intestinal tract of marine shrimp, *L. vannamei*. Therefore, Partenen and Moroz (1999) indicated that, the organic acids are increased activity of proteolytic enzymes and gastric retention time, thus improving digestion. Moreover, the organic acids reduce pH in the stomach, which optimizes conditions for pepsin activity, and increases the digestibility of nitrogen, phosphorus and several minerals (Lückstädt and Mellor, 2011).

Table 21. The interaction effects of different dietary sodium lactate (Na-lacatate) levels on apparent protein digestibility (APD) coefficients and apparent lipid digestibility (ALD) coefficients of experimental diets of *M. rosenbergii* (mean± SD)[A.]

	Control	**Na-lactate**				
		1%	**1.5%**	**2%**	**3%**	**4%**
APD (%)	83.11±1.1[a]	75.32±1.5[b]	73.81±4.1[b]	83.54±0.7[a]	75.44±0.2[b]	84.18±0.3[a]
ALD (%)	62.05±0.4[b]	52.65±1.7[d]	46.89±0.5[e]	47.12±0.6[e]	56.07±1.1[c]	68.08±0.8[a]

Different letters in the same raw display differences according to Duncan's multiple range test (P≤0.05).
[A]Values are the mean of three replicates.

3.2. Histological structure

3.2.1. Experiment I (Nile tilapia)

3.2.1.1. Liver and pancreas

3.2.1.1.1. Control tilapia (Group I)

The liver of the Nile tilapia *O. niloticus*, is located in the pectoral region of the peritoneal cavity of the fish body. The pancreatic exocrine tissue penetrates the posterior part of the liver forming the hepatopancreas. The hepatocytes are arranged in a tubular pattern, the tubules of which are composed, each of six to ten cells surrounding a minute sinusoid.

The liver was primarily composed of polyhedral hepatocytes typically with central or subcentral spherical nucleus, densely stained chromatin margins and a prominent nucleolus. The cytoplasm is homogenous. Venous blood entered the liver caudally from the intestine via the hepatic portal veins and branched into capillaries known as sinusoids (Fig.1). The large sinusoids were lined with reticulo-endothelial cells and lie among the hepatocytes (Fig.2).

The liver contains diffused areas of exocrine pancreatic tissue that are encircled by a thin layer of connective tissue capsule. The portal vein occurs in the centre of the pancreatic structure. The pancreatic acinar cells possess large, pale, round to oval basally located nuclei with prominent nucleoli (Fig.3).

The Nile tilapia *O. niloticus* being one of the most important aquatic organisms is greatly affected by the feed additives and toxicants. This is reflected in the alterations of structure and function of the different organs, tissues and cells (Bruschweiler *et al*., 1996). Therefore, the histopathological changes were more severe in *O. niloticus* than *Tilapia zilli* and *Synodontis schall* in EL-Salam canal (Mohamed, 2003).

The use of the liver as an indicator organ of the nutritional and physiological status in fish is well-known (Hibiya, 1982; Storch and Juario, 1983; Segner and Juario, 1986) such as environmental stressors (Dutta *et al*., 1993) and dietary lipid content (Caballero *et al*., 1999). Hence, it is sensitive to many nutritional compounds and environmental contaminants which accumulate in the liver and exposing it to a much higher levels more than other organs (Heath, 1995). Therefore, Manning *et al*. (2003) indicated that hepatopancreatic tissue of *Ictalurus punctatus* is more sensitive to dietary mycotoxins than renal tissue. Thus, several authors have described liver alterations produced by different nutritional factors. Some authors suggested pathological conditions in livers as result of dietary lipid imbalances (Bautista and De la Cruz, 1988; Watanabe *et al*., 1989).

Previous reports studied the histological structure of the liver and pancreas of Nile tilapia (Caballero *et al*., 1999; Jiraungkoorskul *et al*., 2003; Figueiredo-Fernandes *et al*., 2006, 2007; Peebua *et al*., 2008; EL-Hosseiny, 2009). The liver tissue of Nile tilapia resembles that described for other teleost fish by Saleh and Hamza (1986) on *Tilapia zillii*, Biagianti (1991) on grey mullets, Gonzalez *et al.* (1993) on *Serranus cabrilla*, Dapra *et al*. (2005) and Tusche *et al*. (2011) on *Oncorhynchus mykiss* and Martínez-Llorens *et al*. (2012) on *Sparus aurata*.

3.2.1.1.2. Treated tilapia (Group II)

In the present work, we examined the microscopic anatomy of liver and pancreas of the Nile tilapia *O. niloticus* reared in three levels (2, 3, and 4%) of three kinds of organic acid salts as acidifiers (sodium lactate, calcium lactate, and calcium propionate) after 74 days.

At diet supplement with 2% sodium lactate, the liver tissue of tilapia showed mild fatty degeneration cause to slight vaculation and destruction in

some hepatocytes, also some congestion of the portal vein in the pancreatic tissue (Fig.4). The ratio of vacuoles to hepatocytes increased with increase of sodium lactate's level resulting from fatty degeneration (steatosis); at 3% sodium lactate, the liver tissue exhibited destruction and vascularisation of hepatic and pancreatic tissue, the sinusoidal spaces decreased slightly due to the swelling of the hepatocytes and increased blood cells (erythrocytes) due to low hemorrhage and congestion of blood vessels, (Fig.5). At 4% sodium lactate, we noted hypertrophic hepatocytes as denoted by increasing vascularisation and reduced sinusoidal space lead to unclear cellular contours and only the nucleuses observed (Fig.6). Regarding the pancreatic tissue, severe congestion in the blood vessel, increase destruction and rupture of tissue were observed (Fig.6).

Histologically, liver of Nile tilapia *O. niloticus* treated with calcium lactate is illustrated in figures (7, 8 & 9). At 2% level, the liver exhibited destruction of hepatocytes and ruptures of pancreatic tissue. Also, hemorrhage, decreased sinusoidal space and cytoplasmatic vacuolation due to increased steatosis (Fig.7). At 3% calcium lactate, more histological alterations of liver and pancrease as; necrosis and shrinkage of pancreatic tissue and increased cytoplasmatic vacuolation of hepatocytes which lead to hypertrophic hepatocytes were noted (Fig.8). Whereas, at 4% level, vacuoles increased in the hepatocytes which lead to pyknotic nuclei, congestion of the blood vessels in the pancreatic structures, and rupture of pancreatic tissue due to shrinkage were observed (Fig.9).

In the fish treated with calcium propionate, some abnormal histological alterations of liver and pancreas were observed. At 2% level, both the hepatic and pancreatic structures appeared generally intact except some vacuoles into hepatocytes (Fig.10). On the contrary, at 3% level, increasing vacuoles became noticeable; as well as, atrophic hepatocytes, pancreatic tissue

shrinkage, congestion blood vessels and drastic necrosis were observed (Fig.11). Regarding the 4% level, we showed foci of hypertrophic hepatocytes with enlarged irregular nuclei located at the periphery of the cell, great degeneration of liver and pancreatic tissue due to high level of vacuoles and necrosis, also increased congestion blood vessels (Fig.12).

Observations from this book investigate that all treatments showed vacuoles of the liver and pancreatic tissues of O. *niloticus*, but they increased with increased level gradually. During the experimental period the tissue treated with calcium lactate showed less histopathological changes. Whereas, the tissue of liver and pancreas from fish fed on diet contains sodium lactate and calcium propionate exhibited high histopathological lesions especially at 4% level.

Similar histopathological changes of the liver and pancreas of Nile tilapia were observed in previous reports on the same species. Where, Sanchez *et al*. (1994) and Tuan *et al.* (2002) added aflatoxin B1 (treat infection) to diet of *O. niloticus* at several levels, and they found excess lipofuscin, irregularly sized hepatocellular nuclei, nuclear and cellular hypertrophy and severe hepatic necrosis. Moreover, EL-Hosseiny, (2009) investigated the effects of vitamin C, E, cobalt chloride and their blends as growth promoter of Nile tilapia; he noted congested blood sinusoids, destruction and necrosis of the liver and pancreatic tissues and fatty hepatic degeneration (steatosis).

More or less similar results on other fish species were given by Tucker *et al*. (1997) who observed changes in hepatocytes of red drum (*Sciaenops ocellatus*) fed diets containing menhaden oil and soybean oil. Also, Dapra *et al*. (2005) substituted for fishmeal by plant protein (red kidney bean) in rainbow trout, *Oncorhynchus mykiss*, and that act some histological changes of liver, increase large transparent vacuoles in cytoplasm of hepatocytes and congestion in the blood vessels. In addition, Yamamoto *et al*. (2010) observed

mild abnormalities of the liver of rainbow trout (*O. mykiss*) in a feeding trial with fermented and unfermented soybean meal as fishmeal substitute. Also, Mérida *et al*. (2010) noted small vacuoles and moderate nuclear atrophy of the hepatocytes, while most of the pancreatic acinar cells showed distinct acini and pale nuclei when added sunflower meal to diet of sharpsnout sea bream, *Diplodus puntazzo*. Thus, Tusche *et al*. (2011) conducted to evaluate the effect of potato protein concentrate replacing fishmeal in nutrition of *Oncorhynchus mykiss*; they observed hypertrophy of the liver as denoted by increasing vascularisation and reduced sinusoidal space to a degree. Likewise, Martínez-Llorens *et al*. (2012) used of carob seed germ meal as a substitute for fish meal of gilthead sea bream fingerlings, *Sparus aurata*; the liver sections showed hepatocytes with cytoplasm vacuolization and evident nuclear displacement.

The present findings are in agreement with what was claimed by Hu *et al*. (2013) who substituted of prime steam dried fish meal by animal protein blend might induce hepatic steatosis for Japanese seabss, *Lateolabrax japonicus*. Also, Tan *et al*. (2013) investigated the effects of different dietary rapeseed meal level on grass carp**,** *Ctenopharyngodon idellus*, and the histological examination showed vacuoles in the cytoplasm of hepatocytes and parenchymal necrosis of liver.

Conflicting reports are found in the literature. Manning *et al*. (2003) noticed more lesion of pancreatic tissue when added Ochratoxin A (mycotoxins) to diet of channel catfish, *Ictalurus punctatus,* and observed absence of pancreatic tissue and presence of melanomacrophage centers in normally pancreatic regions in the area surrounding a portal vein of the liver is probably indicative of cellular breakdown of exocrine pancreas. While, Evans *et al*. (2005) reported that there is no differences during using heat-treated raw soybean meal or non-heat for fed catfish, *Ictalurus punctatus* in liver and pancreatic tissues. Also, Valentim-Zabott *et al*. (2008) added

Homeopatila RS diet (as vital energy of the organism) of *O. niloticus*; it caused less hepatic lipid inclusions due to less vacuolization. On other hand, other studies investigated that astaxanthin (Segner *et al*., 1989) and Sangrovit® (Rawling *et al*., 2009) had positive effects on the hepatic function and histology as feed additive for *O. niloticus*.

The vacuolization might indicate an imbalance between the rate of synthesis of substances in the parenchymal cells and the rate of their release into the systemic circulation (Gingerich, 1982). This is led to the cellular degeneration and destruction in the liver and pancreatic tissues; and may be due to the intravascular haemolysis observed in the blood vessels with congestion of blood (Mohamed, 2001). Thus, Bell *et al*. (1995) observed a high degree of vacuolation due to lipid deposition in livers of turbot (*Scophthalmus maximus*), and this is related with lipid digestability (Verreth *et al*., 1994) and resulting from fatty degeneration (steatosis) (Spisni *et al*., 1998). This is in agreement with the present work; as well, the best apparent lipid digestibility (ALD) was observed with calcium lactate and showed less alternation in the liver and pancreatic tissue. Therefore, Partenen and Moroz (1999) and Hossain *et al*. (2007) claimed that organic acids improved the apparent digestibility protein in growing pigs, which might stimulate pancreatic secretions; also our present results improved the apparent protein digestibility than control.

Segner and Witt (1990) found that the increase of lipid in liver of turbot *S. maximus* after the start of weaning may only be due to a change of feed and can be considered as an expression of a well-fed status rather than a pathological syndrome. Likewise, Mosconi-Bac (1987) and Fontagné *et al*. (1998) suggested that the presence of numerous and voluminous lipid droplets in hepatocytes may be a physiological response to lipid excess and may not be related to nutritional disorders. Therefore, represents energy storage but not a

pathological situation. In addition, Caballero *et al*. (2004) demonstrated that this hepatocyte vacuolization of *Sparus aurata* can measure the subtle changes in distribution and content of stored nutrients in the hepatocytes, such as glycogen and lipid. Thus, Ghazaly (1994) suggested that the depressed levels of lactic acid of catfish (*Clarias lazera*) led to elevate glycogen level and induce cytotoxicity in liver with acidic levels of pH; this is reflects some disturbances of carbohydrate metabolism. Hence, in the present work we observed high histopathological changes at high levels of organic acid salts as acidifiers.

Several authors have reported that the hepatonuclear size can be used as an indicator of the nutritional condition of fish (Escaffre and Bergot, 1986; Segner and Braunbeck, 1988; Strüssmann and Takashima, 1990; Caballero *et al*., 1999; Jiraungkoorskul *et al*., 2003). This is in agreement with the present work, where our results showed pyknotic nuclei at 4% all organic acid salts used as acidifiers and it is reflecting alteration in fatty acid metabolism, thus being signs of true nutritional pathology (Ghittino, 1978; Mosconi-Bac, 1987) and increased metabolic activity (Braunbeck *et al*., 1990).

Observation from this book showed slight necrosis in the liver and pancreatic tissue of *O. niloticus* at 4% of sodium lactate and 3% and 4% of calcium propionate. The present findings may be explained by the fact that the necrosis observed in the liver and pancreatic tissues is attributed to the destruction and degeneration of hepatic and pancreatic tissues and caused invasion infiltration of leucocytes resulting in the dissolution of the hepatocytes (Ram and Singh, 1988; Jiraungkoorskul *et al*., 2003). And these changes are often indicator to severe alternation of tissue (Myers *et al*., 1987). Moreover, several reports investigated the necrosis and pyknosis in the liver and pancreatic tissue of some fish after treating of pesticides (Gill *et al*., 1990; Dutta *et al*., 1993; Cengiz and Unlu, 2006) and with pollutions (Hemmaid and

Kaldas, 1994; Abdelmeguid *et al*., 1999; Fathallah, 2001; Bukhari *et al*., 2012). The present results showed slight necrosis at 4% of sodium lactate, calcium lactate and 3% and 4% calcium propionate.

The present result showed that 2% of all organic acid salts used as acidifiers are more suitable level than 3% and 4%. 2% proved to be significantly more convenient to Nile tilapia than other levels as growth promoter. Although, 4% sodium lactate recorded higher growth rates.

Fig.1: Photomicrograph of a section of the liver of untreated Nile tilapia *O. niloticus* showing normal hepatocytes in liver tissue, (H&E).

Fig.2: Photomicrograph of a section of the liver of untreated Nile tilapia *O. niloticus* showing large sinusoids were surrounding by reticulo-endothelial cells which lie among the hepatocytes, (H&E).

Fig.3: Photomicrograph of a section of the liver and pancreas of untreated Nile tilapia *O. niloticus* showing pancreatic tissue, (H&E).

Fig.4: Photomicrograph of a sectionof the liver and pancreas of Nile tilapia *O. niloticus* treated with 2% sodium lactate showing slight vaculation, destructed hepatocytes and some congestion of the portal vein in the pancreatic tissue, (H&E).

H= hepatocyte, N= nucleus, S= sinusoid, Bc= blood cell, Bv= blood vessel, Rc=reticulo-endothelial cells, Pt= pancreatic tissue, Pc= pancreatic acinar cell, Pv= portal vein, V= vacuole, S= sinusoid.

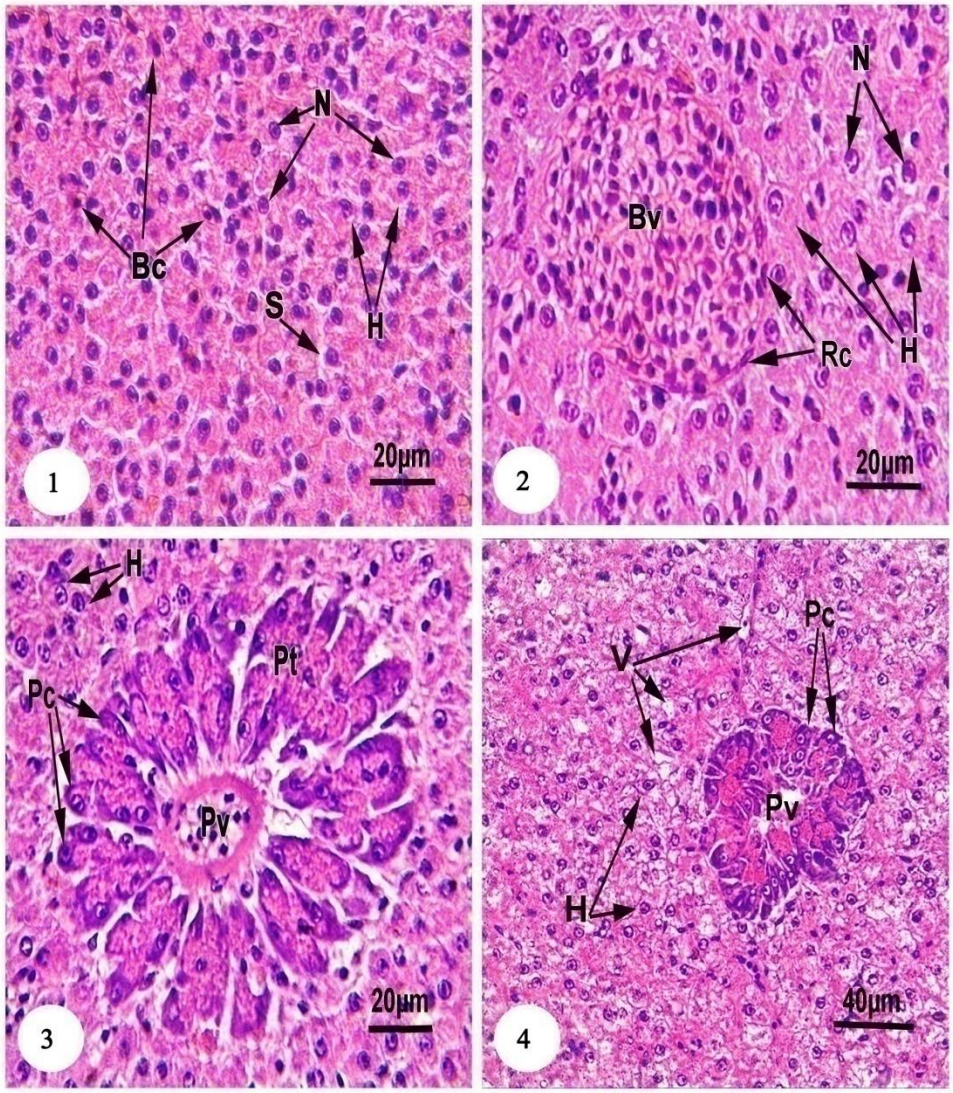

N
Bc
S
H
20µm
1
N
Bv
Rc
H
20µm
2
H
Pt
Pc
Pv
20µm
3
Pc
V
Pv
H
40µm
4

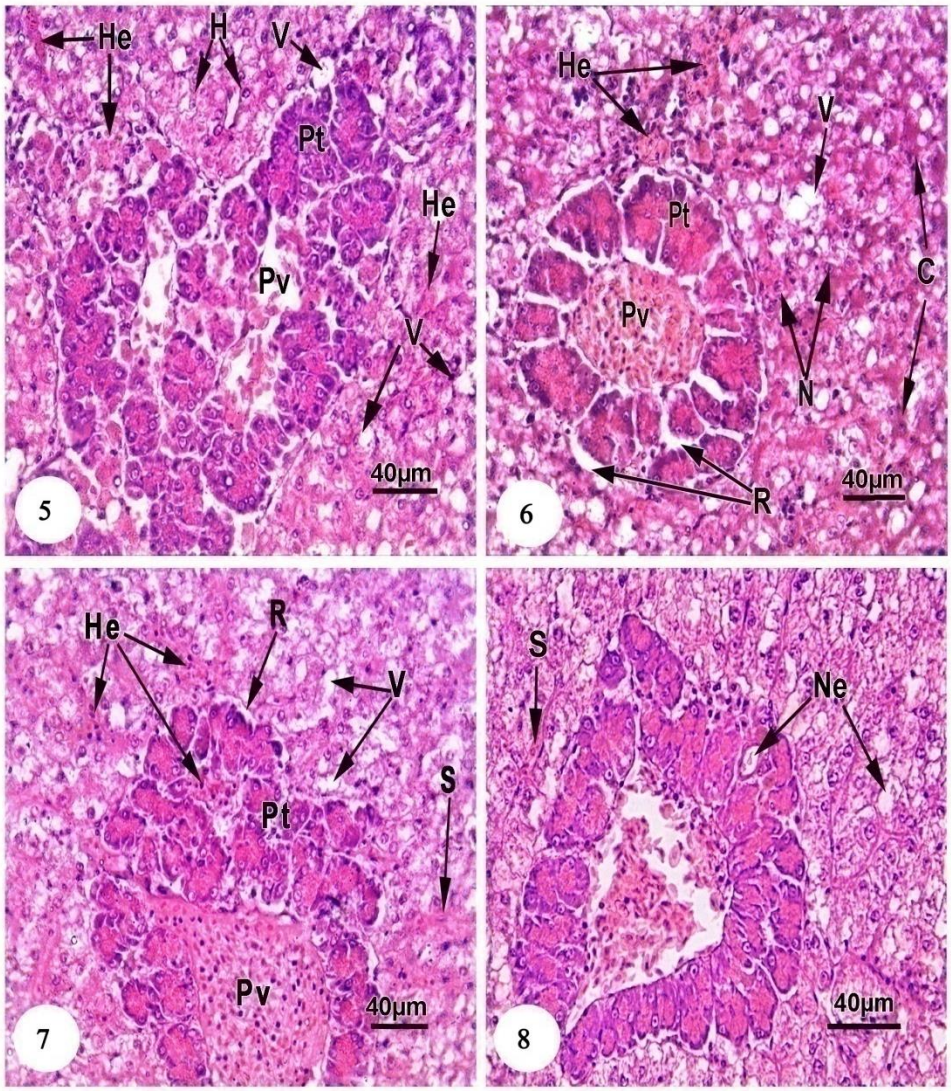
Fig.5: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 3% sodium lactate showing vacuolation and swelling in the hepatocytes and destruction of pancreatic tissue, (H&E).

Fig.6: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 4% sodium lactate showing increased vacuoles in the liver tissue, severe congestion in the blood vessel and rupture of pancreatic tissue, (H&E).

Fig.7: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 2% calcium lactate showing hypertrophic hepatocytes, cytoplasmatic vacuolation and ruptures of pancreatic tissue, (H&E).

Fig.8: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 3% calcium lactate showing increased cytoplasmatic vacuolation of hepatocytes, necrosis and shrinkage of pancreatic tissue, (H&E).

H= hepatocyte, N= nucleus, Pt= pancreatic tissue, Pv= portal vein, V= vacuole, He= hemorrhage, R= rupture tissue, S= sinusoid, C= congestion, Ne= necrosis.


He
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S
Pt
Pv
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7
S
Ne
40µm
8

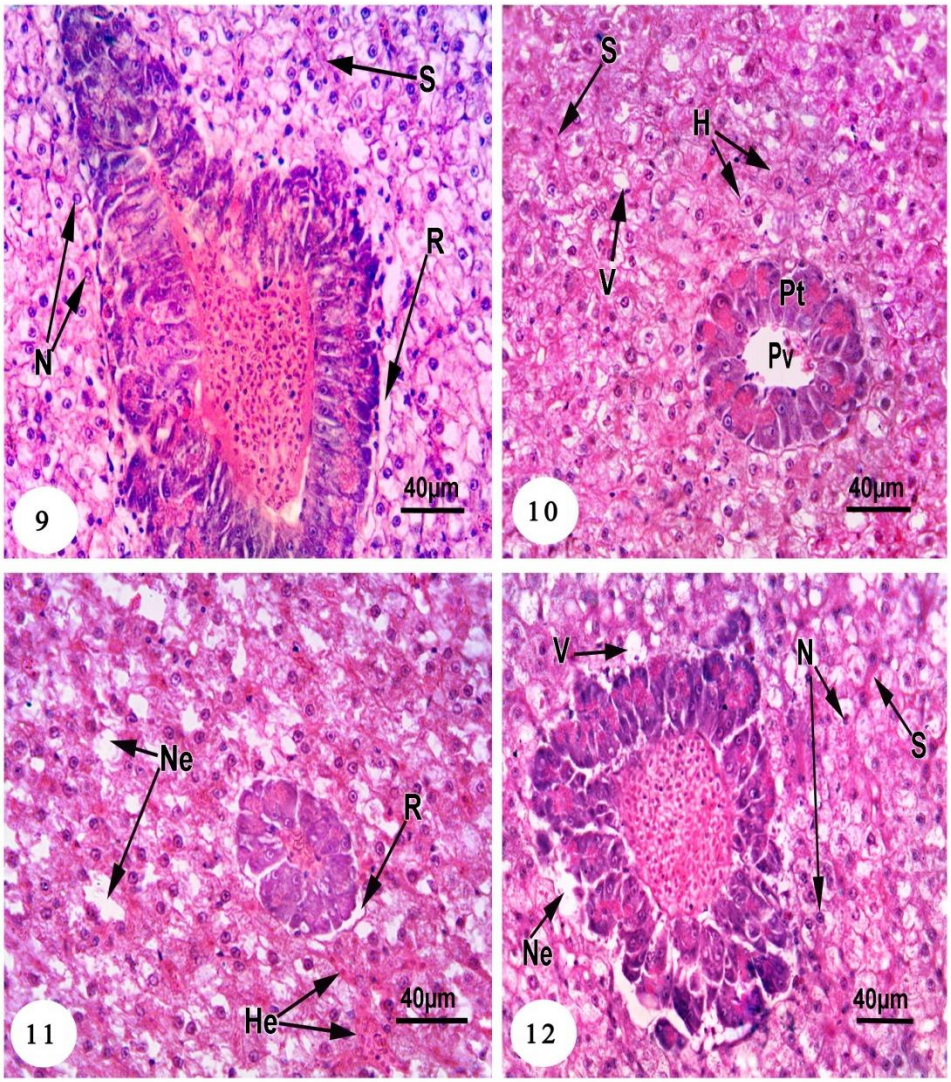
Fig.9: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 4% calcium lactate showing pycnotic nuclei of the hepatocytes and congestion of the blood vessels in the pancreatic structures, (H&E).

Fig.10: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 2% calcium propionate showing slight vacuolation into hepatocytes and intact pancreatic tissue, (H&E).

Fig.11: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 3% calcium propionate showing increasing vacuoles of hepatocytes, pancreatic tissue shrinkage and congestion of blood vessels, (H&E).

Fig.12: Photomicrograph of a section of the liver and pancreas of Nile tilapia *O. niloticus* treated with 4% calcium propionate showing enlarged irregular nuclei located at the periphery of hepatocytes and great degeneration of liver and pancreatic tissue, (H&E).

H= hepatocyte, Pt= pancreatic tissue, Pv= portal vein, N= nucleus, S= sinusoid, R= rupture tissue, Ne= necrosis, V= vacuole, He= hemorrhage.


S
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N
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9
S
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10
Ne
R
He
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11
V
N
S
Ne
40µm
12

3.2.1.2. Gonads

3.2.1.2.1. Control tilapia (Group I)

3.2.1.2.1.1. Ovary

The ovaries of the Nile tilapia, *O. niloticus* are paired elongated structures, and extend in the abdominal cavity from the posterior to the anterior. The ovaries are nearly equal in size and slightly separated from each other; whereas the two oviducts unit together forming one common oviduct that leads to the genital pore.

According to the degree of development and accumulation of the yolk, the stages of the oocytes comprise two phases, the growth phase (previtellogenesis) and the maturation phase (vitellogenesis).

The previtellogenesis consists of chromatin nucleolus stage (oogonia), early and late perinucleolus stages (EpO and LpO). The chromatin nucleolus stage (oogonia), where the oocyte had weakly basophilic cytoplasm and more basophilic nucleus with darkly staining nucleoli (Fig.13). Early and late perinucleolus stages whereas the first stage (EpO) is characterized by light stain, large centrally nucleus with one or more periphery nucleoli, it had a dark cytoplasm and surrounded by a thin follicular epithelial layer (Fig.13), but the second stage (LpO) have large size and distinguished by the less basophilic cytoplasm, nucleus became more round shape, in addition to the numerous observed nucleoli at the peripheral of nucleus membrane and it is surrounded with a follicular wall consisting of an outer connective tissue layer and an inner squamous or flattened epithelial cells layer (Figs.13 & 14).

The maturation phase (vitellogenesis) includes four stages, yolk vesicle, primary yolk, secondary yolk and tertiary yolk. The yolk vesicle is main characterized by the presence of yolk vesicles “or lipid droplets’’ as unstained vacuoles scattered of cytoplasm (Fig.14). The cytoplasm of this stage is homogeneous and weakly basophilic, the nucleus has one or more

nucleoli. Thus, the follicular wall of the oocyte is differentiated into an outer theca externa (connective tissue layer) and an inner theca interna (squamous epithelial layer).

The primary yolk stage is distinguished by the presence of acidophilic yolk granules at the periphery of the slightly basophilic cytoplasm, the nucleus is peripheral and irregular shape and it has one or more nucleoli at the margin; while secondary yolk stage, acidophilic yolk granules and the lipid vesicles are increased in number, the nucleus is eccentric in position and irregular shape and it has and one or more nucleoli at the margin too (Fig.14). The follicular wall of the primary and secondary yolk stage is differentiated into two layers; an outer connective tissue layer, an inner thin layer of epithelial cells.

The Tertiary yolk stage has no limited nuclear membrane, it has amoeboid nucleus and the yolk granules are numerous, densely packed and united together to form fluid-like masses inside the oocytes (Fig.14). The follicular wall is now well developed and more prominent. The layers of follicular wall are from outside inwards, the follicular epithelial layer (theca folliculi) , which is followed by a jelly-like layer, the zona radiata which has become thinner due to the increase in size of the oocyte, and a theca interna, which is a thin layer consisting of squamous epithelial layer (Fig.15).

Some vacuolated or mature follicles may suddenly cease development and undergo atresia after ovulation. These atretic follicles show remarkable structural variations and can be divided into three general stages. The first stage is characterized by deterioration of the nucleus and yolk materials. In the second stage, the nucleus completely disappeared, the zona radiata gradually deteriorated, and yolk materials aggregated under the zona radiate. In the last stage, the zona radiata becomes completely disappeared and the cytoplasm did not contain any yolk materials and it was lightly stained (Fig.16).

3.2.1.2.1.2. Testis

The testes of the Nile tilapia, *O. niloticus*, located in the body cavity ventral to the kidneys and extend anterior to the beginning of the cardiac stomach. They are white yellowish elongated organs. The two testes are united at their seminal ducts near their ends forming a short common duct leading to the urinogenital pore in front of the anal fin and behind the anal opening.

The testis is a compound tubular gland surrounded with a dense thick connective tissue capsule, the tunica albuginea, and composed of a large number of tubules, the seminiferous tubules. The connective tissue of the tunica albuginea extends in between the seminiferous tubules to form the intertubular tissue containing leydig cells (the interstitial cells). The seminiferous tubules appear in transverse sections as irregular, rounded, oval or elongated compartments and contain spermatogenic cells (Fig.17).

Spermatogenesis includes five stages, spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa.

The spermatogonia are found in nests (spermatogonial cysts) and represent the largest spermatogenic cells. They are rounded or slightly ovoid in shape and characterized by large spherical, darkly stained centrally located nuclei; each nucleus contains one conspicuous nucleolus with coarse chromatin granules close to the nuclear membrane (Fig. 17).

The primary spermatocytes are more or less rounded in shape and possess faintly stained cytoplasm, whereas the nuclear chromatin material appears as a coarse reticulum in the centre of the nucleus. The secondary spermatocytes are smaller than the primary ones and possess less cytoplasm with small densely stained nuclei. The spermatids are smaller in size than the secondary spermatocytes and appear as dense clusters. Their nuclei are darkly stained and nearly occupy the whole cell (Fig.17).

The spermatozoa appear as aggregations in the centre of the seminiferous tubules. They are the smallest germ cell type and have intensely stained slightly rounded heads, while faintly stained tails therefore disappearing (Fig.17).

Moreover, the same gonads of the same species (*O. niloticus*) showed similar description and was subsequently confirmed when studied by Moharram and Ebiary (2003), Jegede and Fagbendro (2008a, b) and EL-Hosseiny (2009). Similar results were reported by other authors such as Kjesbu and Keyvi (1989), Palmar *et al.* (1995), Mojazi *et al.* (1996), Assem (1999), Mamuris *et al.* (1998), EL-Gohary (2001), EL-Gamal (2003) and Tayel (2003) on other teleost fish.

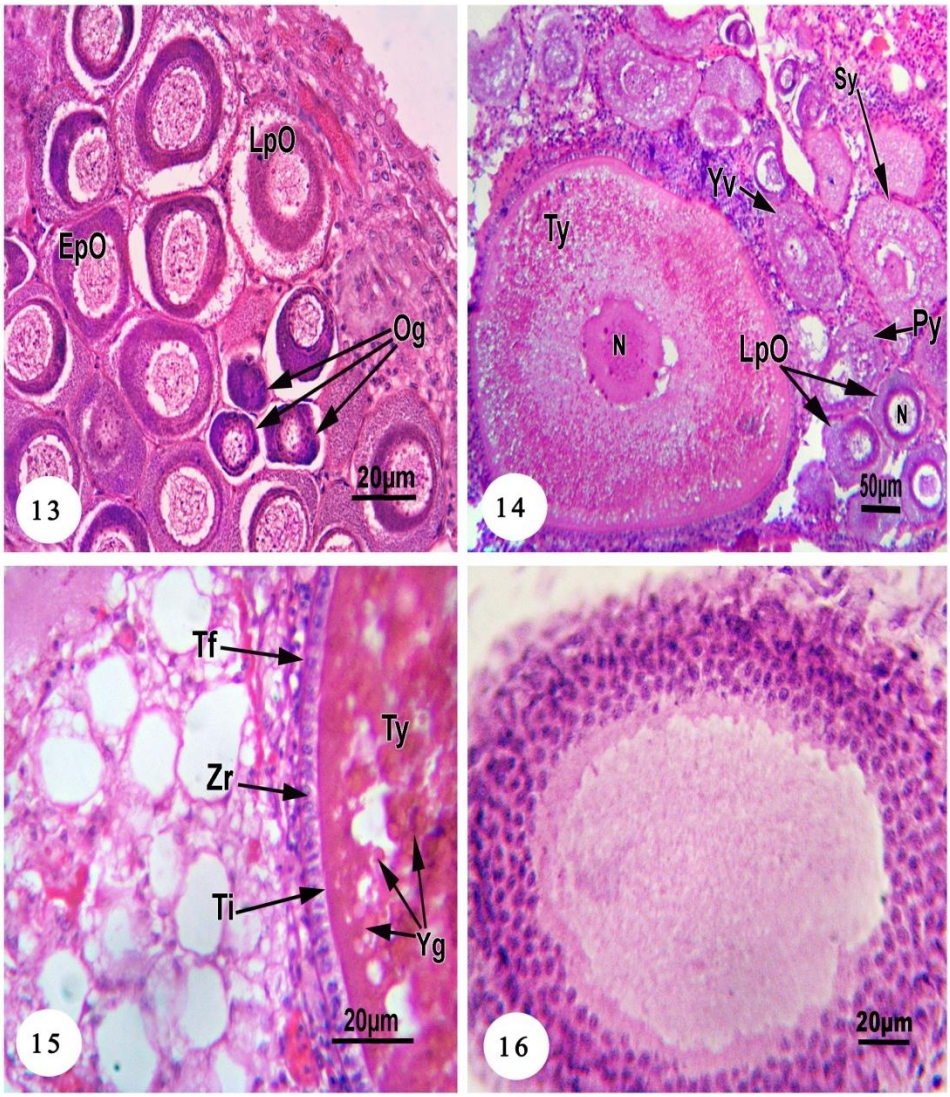
Fig.13: Photomicrograph of a T.S. of the ovary of normal Nile tilapia, *O. niloticus* showing previtellogenic oocytes, (H&E).

Fig.14: Photomicrograph of a T.S. of the ovary of normal Nile tilapia, *O. niloticus* showing late perinucleolus stage and vitellogenic oocytes, (H&E).

Fig.15: A highly magnified part of the same as in Fig.14 showing tertiary yolk stage and the layers of follicular wall, (H&E).

Fig.16: Photomicrograph of a T.S. of the ovary of normal Nile tilapia *O. niloticus* showing 3rd stage of atresia, (H&E).

Og= oogonia, EpO= early perinucleolus oocyte, LpO= late perinucleolus oocyte, N= nucleus, Yv= yolk vesicle stage, Py= primary yolk stage, Sy= secondary yolk stage, Ty= tertiary yolk stage, Yg= yolk granule, Ti= theca interna, Zr= zonaradiata, Tf= theca folliculi.



3.2.1.2.2. Treated tilapia (Group II)

After 74 days of treatment of *O. niloticus* with three levels (2, 3 and 4%) of three kinds of organic acid salts as acidifiers (sodium lactate, calcium lactate, and calcium propionate). No abnormalities were detected in the tissue of ovary. The gland exhibited its normal pattern showing normal oocytes.

Concerning the testis, clear trend exhibited a variety of histological alternations. At diet supplement with 2% sodium lactate, the testis showed slight degeneration of spermatogonia, thinness tunica albuginea and some erytherocytes were visible between the seminiferous tubules (hemorrhage) (Fig.18). At 3% sodium lactate, the testis revealed increase degeneration of spermatogonia, vacuoles in cellular elements of some seminiferous tubules, increase hemorrhage, thinness tunica albuginea and lesser number of sperms indicating lack of active spermatogenesis (Fig.19). In fish fed on diet supplement with 4% sodium lactate, the testis showed more histological changes as severe testicular tissue atrophy, extensive hemorrhage, necrosis, pyknosis and tissue disintegration led to unclear types of spermatocyte and cellular contours (Fig.20).

The histological structure of testis's *O. niloticus* at 2% calcium lactate as feed additive; exhibited the seminiferous tubules are normal and full of spermatocytes, also the spermatid and spermatozoa are observed in the centre of these tubules (Fig.21). While at 3% level, slight hemorrhage but normal seminiferous tubules and spermatocytes were observed (Fig.22). Regarding, at 4% calcium lactate, hemorrhage, degenerative and necrotic change in the cellular elements of some seminiferous tubules were observed (Fig.23&24).

Diet supplement with calcium propionate, exhibited some marked histological changes of testis. The fish fed on 2% calcium propionate showed normal spermatocytes excluding many blood vessels were packed with erythrocytes (hemorrhage) were visible between seminiferous tubules, this lead to blood vessels enlargment (Fig.25). At 3% calcium propionate,

hemorrhage, destruction of leydig cells and multiple areas of necrosis due to degeneration of seminiferous tubules and spermatocytes were observed in the testis of the treated fish (Fig.26). Concerning, 4% calcium propionate, remarkable malformation and disruption of seminiferous tubules, degeneration of leydig cells and increase hemorrhage and necrosis lead to pyknosis were noticed (Fig.27). Moreover, some seminiferous tubules revealed a lesser number of sperm due to failure of spermatogenesis.

The fish gonads, a possible indicator of physiological disturbances, have rarely been studied with relation to the possible histopathological effects of foods. The present findings are similar with results reported by other authors. Thus, EL-Hosseiny (2009) showed oocytes at different stages in normal state and ready for ovulation and some atretic follicles at the late stages of ovulation of *O. niloticus* fed on vitamin C, E and cobalt chloride. Likewise, Celik and Altun (2009) did not observe any negative effects on ovarian tissues of Nile tilapia fed on vitamin E levels. This is in agreement with the present results, where showing normal ovarian tissue at different organic acid salts and their levels.

Several conflicting reports are found in the literature; Cumaranatunga and Thabrew (1989), Moharram and Ebiary (2003), Mohamed (2003), Jegede and Fagbendro (2008a) in *O. niloticus* and Jegede and Fagbendro (2008b) in *Tilapia zillii*. They studied the effects of some feed additives on ovarian tissue and claimed histological abnomalities such as pyknosis and necrosis as histopathological changes.

The present results are in agreement with what investigated by Mohararm and Ebiary (2003) who added different levels of active yeast (*Saccharomyces servisiaein*) to diet of *O. niloticus* and observed many blood vessels were packed with erythrocytes between seminiferous tubules at lower levels; But they showed the histopathological changes of testicular tissue more

lesion than the present work including, pyknosis and complete degeneration of primary and secondary spermatocytes, of the seminiferous tubules wall and all the intermediate stages were not observed between spermatogonia and spermatozoa.

The present work of testicular tissue at different organic acid salts as acidifiers and levels showed fewer lesions than what found in some previous reports. Jegede and Fagbendro (2008a) showed some pathological changes of *O. niloticus* fed on pawpaw (*Carica papaya*) seed meal (fertility control agents) at several concentrations, thus, who observed atrophy of seminiferous tubules and absent of spermatids. Likewise, Jegede and Fagbendro (2008b) added neem (*Azadirachta indica*) leaf meal (antimicrobial) at different levels to a basal diet of *Tilapia zillii* who revealed alteration in testicular architecture such as cystic seminiferous tubules and severe testicular atrophy.

The present results are more similar with EL-Hosseiny (2009) who added vitamin C, E and cobalt chloride to diet of *O. niloticus* and noticed some histopathological changes of testis such as destruction of the leydig cells, degeneration of some seminiferous tubules and spermatogenic cells and thin tunica albuginea; and some treatment showed intact seminiferous tubules with different stages of spermatocytes.

On the other hand, similar results were reported by Mohamed (2003) who studied the effect of some pollutants on the histological features of testis of Nile tilapia living in the El-Salam canal, and observed degenerative and necrotic changes of some seminiferous tubules.

The present results indicate that high level (4%) of all types organic acid salts used as acidifiers, caused testicular histopathological effects.

Fig.17: Photomicrograph of a T.S. of the testis of normal Nile tilapia *O. niloticus* showing different spermatogenic cells, seminiferous tubules and tunica albuginea (H&E).

Fig.18: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 2% sodium lactate showing slight degeneration of spermatogonia and a little hemorrhage between seminiferous tubules, (H&E).

Fig.19: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 3% sodium lactate showing increase degeneration of spermatogonia, vacuoles and increase hemorrhage, (H&E).

Ta= tunica albuginea, Sg= spermatogonia, St= seminiferous tubules, Ps= primary spermatocyte, Ss= secondary spermatocyte, Sd= spermatids, Sz= spermatozoa, Lc= leydig cells, C.T= connective tissue, He= hemorrhage, V= vacuoles.

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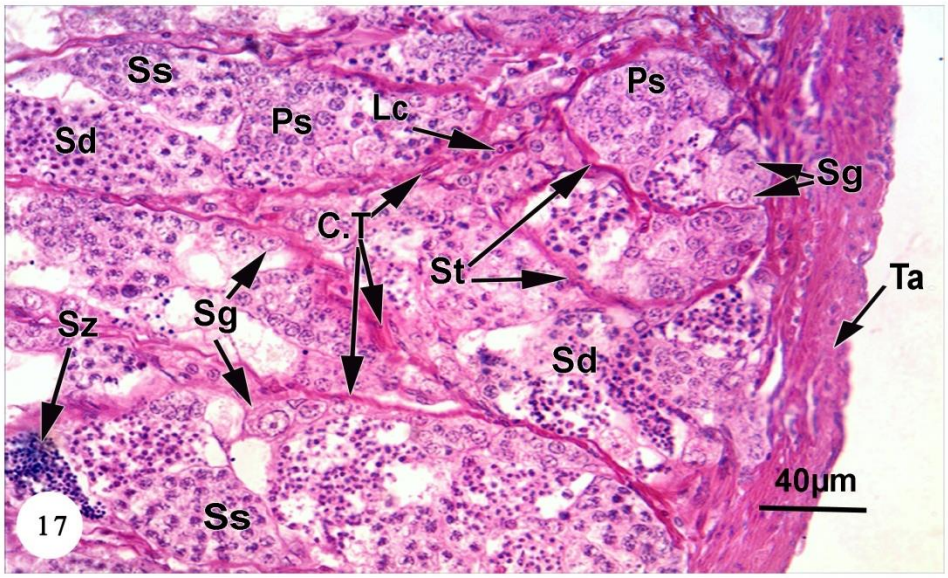
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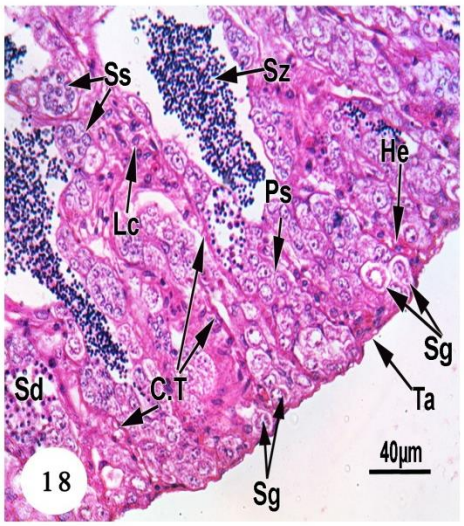
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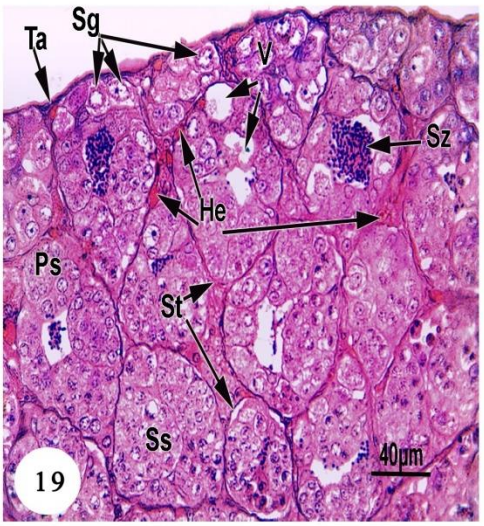
Ta
Sg
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Ps
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Ss
40µm
19

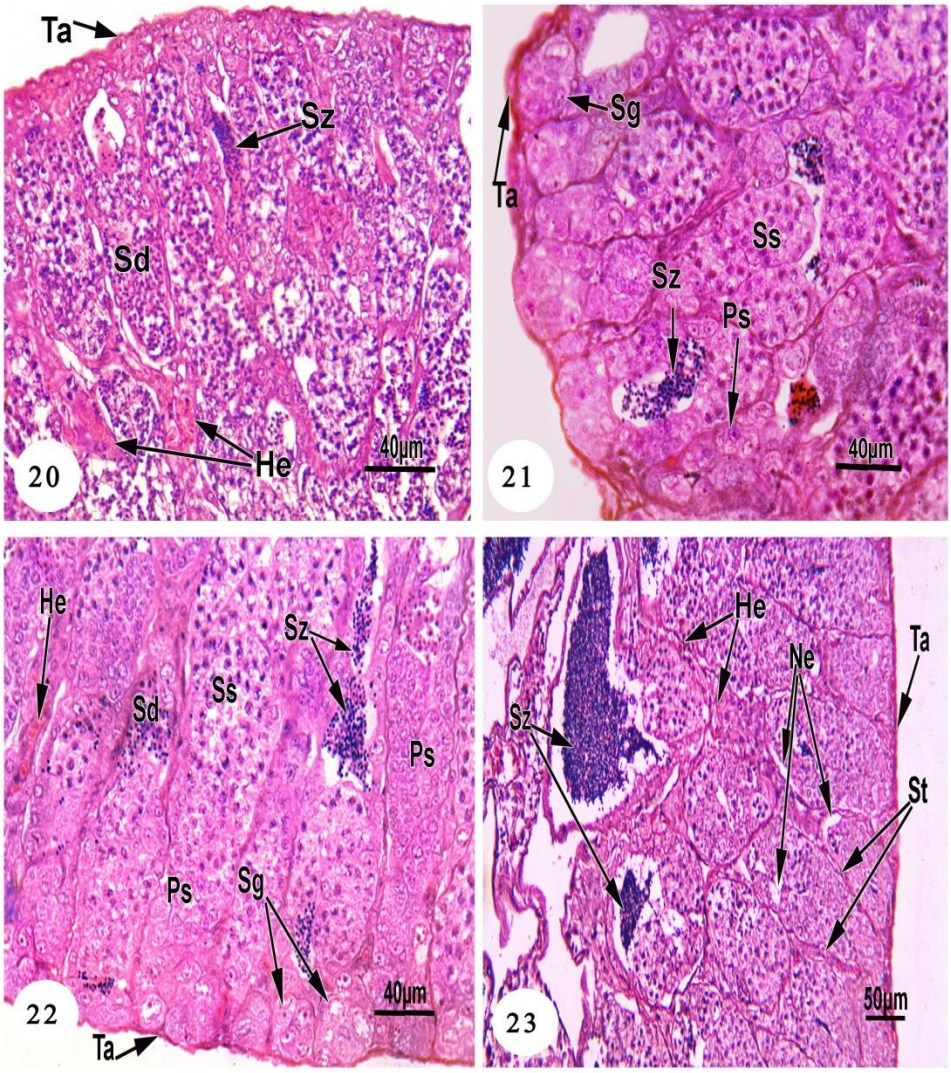
Fig.20: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 4% sodium lactate showing extensive hemorrhage, necrosis and pyknosis, (H&E).

Fig.21: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 2% calcium lactate showing normal seminiferous tubules, (H&E).

Fig.22: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 3% calcium lactate showing slight hemorrhage between seminiferous tubules, (H&E).

Fig.23: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 4% calcium lactate showing necrotic change in the cellular elements of some seminiferous tubules, (H&E).

Ta= tunica albuginea, St= seminiferous tubules, Sg= spermatogonia, Ps= primary spermatocyte, Ss= secondary spermatocyte, Sd= spermatids, Sz= spermatozoa, He= hemorrhage, Ne= necrosis.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Sg
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22
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Ne
Ta
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St
50µm
23

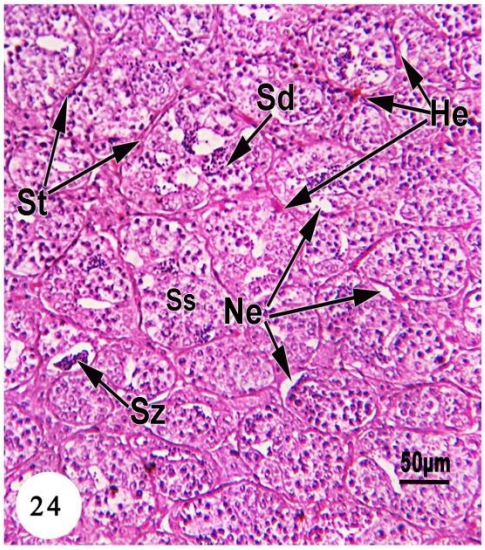
Fig.24: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 4% calcium lactate showing crowding of the spermatocytes into seminiferous tubules, (H&E).

Fig.25: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 2% calcium propionate showing slight hemorrhage between seminiferous tubules, (H&E).

Fig.26: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 3% calcium propionate showing destruction of the seminiferous tubules and spermatocytes, (H&E).

Fig.27: Photomicrograph of a T.S. of the testis of Nile tilapia *O. niloticus* treated with 4% calcium propionate showing malformation of the seminiferous tubules, (H&E).

Ta= tunica albuginea, Sg= spermatogonia, St= seminiferous tubules, Ps= primary spermatocyte, Ss= secondary spermatocyte, Sd= spermatids, Sz= spermatozoa, Lc= leydig cells, He= hemorrhage, Ne= necrosis.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Sz
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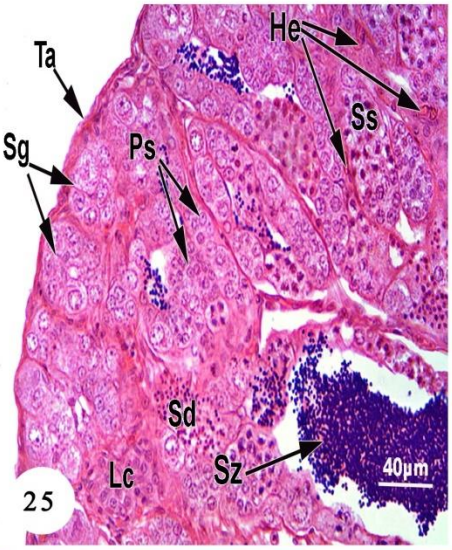
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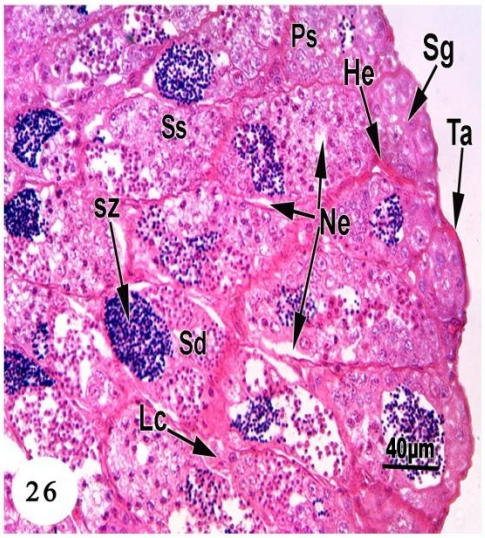
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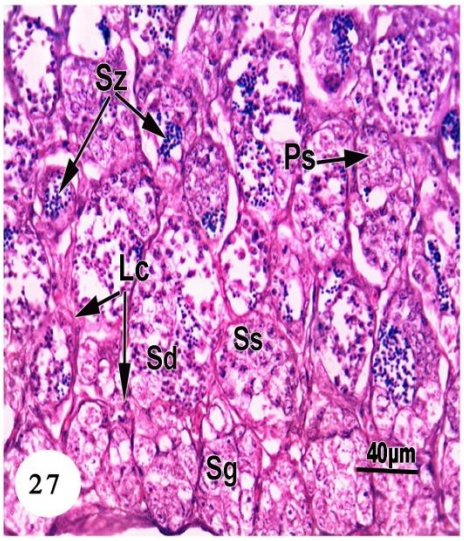
Sz
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27

3.2.2. Freshwater prawn (*Macrobrachium rosenbergii*)

3.2.2.1. Hepatopancreas

3.2.2.1.1. Control prawn (Group I)

The hepatopancreas (mid gut gland or digestive gland) of the normal control *M. rosenbergii* is a bilobed organ (right and left of the gut). It opens into the midgut by collecting short ducts which finally terminate in the antechamber, and it has alight beige color. These two lobes are not separate from each other, surrounded the mid gut with connective tissue. As a result, the hepatopancreas can be subdivided into proximal and distal regions relative to the distance from the main digestive tract.

The gland is made of a great number of oval or circular acini (tubules). Each acinus is lined with simple epithelium and separated from the neighboring one by a thin sheet of connective tissue, which leads into collecting ducts that finally terminate in the antechamber of the mid gut. The cells constituting the wall of tubule are arranged around a wide irregular lumen. The cross section of the lumen has a star-like shape (Fig.28). The lining epithelium of the tubules composed of four main types of cells. Embryonic or embryonalzellen cell ‘‘E-cell’’. Its shape varies between cuboidal and columnar epithelial cell, with rounded to oval nuclei each containing conspicuous nucleolus. The nucleus of E-cells is in the middle region of cytoplasm and predominates in distal tubule tips (Fig.28). Restzellen cell ``R-cell`` is columnar shape, has basal rounded nuclei, and bear an apical microvillar border (Fig.29). It contains variable sized small lipid vacuoles. Lipid droplets have been found only in R-cells (Fig.29). Blasenzellen cell ‘‘B-cell’’ contain a giant single large secretory vesicle in the center of the cells and occupying most of the cell size (Figs.28 & 29). Plasma membrane invaginations in some of the B-cells were also observed, this may be due to absorption of materials from the lumen (Fig.30). The nucleus of B-cell is

lateral and flat to oval in shape. Fibrillenyellen or fibrous cell ‘‘F-cell’’ is spindle shape. It is found between the B-cells and F-cells. In these cells, the nucleus is located at the middle of the cell; the cytoplasm is non-vacuolated and darkly stained with H&E (Figs. 28 & 29). The E-cells are dominated in distal tubule tips of the hepatopancreas, while R-cells, F-cells and B-cells are found frequently in its intermediate and proximal regions.

Histologically, the general structure of hepatopancreas of *M. rosenbergii* is in agreement with similar studies on other decapod crustaceans (Storch *et al.*, 1984; Vogt *et al.*, 1985; Aly, 2000; Sayed, 2002; Schultz, 2005; Sousa *et al*., 2005; Bray *et al.*, 2006; Li *et al*., 2008; Calvo *et al.*, 2011; Xiao *et al*., 2014). More or less similar results were reported by authors, Aly (2000) and Sayed (2002) who worked on crayfish *Procambarus clarkii*. They suggested the presence of two main types of cells depended on the function, digestive and secretory cells. The digestive cells included R and B-cell, and the secretory cell is F-cell. Also, Shyamasundari *et al*. (1990) described only two types of cells, a type with vacuoles and a type without vacuoles, in hepatopancreas of *Nerocila serra* (isopod). While, Nunes *et al*. (2014) identified in hepatopancreas of *fantepenaeus brasiliensis* (pink-shrimp) five types of cells; B (vesicular), E (embryonic), F (fibrillar), M (basal) and R (resorptive).

3.2.2.1.2. Treated prawn

3.2.2.1.2.1. Experiment II (Group II)

In the present work, we examined histologically the hepatopancreas of freshwater prawn *M. rosenbergi* reared in different levels (2, 3 and 4%) of three kinds of organic acid salts as acidifiers (sodium lactate, calcium lactate, and calcium propionate) after 74 days.

In the cross section of the hepatopancreas the lumen of acini is star-like shape of all treatments with sodium lactate as in normal tissue. At 2% sodium lactate, structural disorganization of the tubules, abnormalities of the B and R-cell and atrophy of F-cell were observed (Fig.31), while the connective tissues surrounding the tubules were normal. At 3% sodium lactate, the swollen of F-cell, crowding of the vacuoles and nuclei lead to made difficult identification of cells (Fig.32). The degeneration of the hepatopancreatic cells cause to exhibit few slough in the lumen; the connective tissues and E-cell surrounding the hepatopancreatic tubules were degenerated. Under the treatment of 4% sodium lactate these was a hypertrophy vacuoles of R-cells, narrow lumen, some sloughing, and degeneration of the F-cells and the connective tissues surrounding the hepatopancreatic tubules (Fig.33)

At calcium lactate treatment, all levels exhibited gradual tissues abnormality. Degeneration of R and B-cells were observed in treatment at 2% of calcium lactate during work period. Also slough into the lumen and adjoining with microvilli were exhibited (Fig.34). Thus, deformation of B-cells and R-cells, and F-cells were noticed (Fig.34). At 3% of calcium lactate the connective tissue damage, narrow lumen and malformation of B, R and F-cells were demonstrated (Fig.35). Regarding the 4% calcium lactate level, more lesion of the hepatopancreatic tissue was exhibited (Fig.36). Also, narrow lumen, shrinking of connective tissue due to swollen cells, degeneration and disintegration of hepatopancreas, necrosis and dense materials in vacuoles were observed.

The hepatopacreatic tissue treated by calcium propionate showed less lesion compare with other acidifiers (sodium lactate and calcium lactate). At 2% of calcium propionate, slight increase of R-cell vacuoles and slight decrease of B-cell numbers were noted (Fig.37). As well as, at 3% calcium

propionate, slight cell degeneration, few sloughs into the lumen, slight shrinking of connective tissue and reduction of lipid droplets storage indicated by markedly reduced tubule epithelial cell height were observed (Fig.38). Regarding the treatment by 4% of calcium propionate, enlarged of vacuoles, slight changing of F-cells shape, increase shrinking of connective tissue, and narrowing of the lumen due to increase of tubule epithelial cell height were exhibited (Fig.39).

3.2.2.1.2.2. Experiment III (Group III)

In this experiment, we studied the hepatopancreas of freshwater prawn *M. rosenbergii* reared in five levels of sodium lactate (1, 1.5, 2, 3 and 4g/kg of diet).

Treatment of 1% sodium lactate, for 103 days, the hepatopancreas revealed little degeneration of the large secretory vesicle of B-cells lead to exhibit dense materials in vacuoles; and reduced of lipid vacuoles of R-cells (Figs.40& 41). Also, the lumen of tubules have some slough as a result of damage of the tubules epithelium (tissue debris), and lead to an enlarged tubular lumen in the distal portion (Fig.40), while in the proximal portion still narrow (Fig.41). The connective tissue surrounding of hepatopancreas tubule was degenerated and shrinked (Fig.41). Clearly, the destruction of hepatopancreatic cells is increased in the proximal than from the distal portion.

At the 1.5% sodium lactate, changing of the shape of all types of cells, very narrow lumen due to swollen of B, R and F-cells, increased and enlarged vacuoles were observed (Fig.42).

At 2% sodium lactate, more accumulation of vacuoles, damage of hepatopancreatic tubules and connective tissue were observed (Fig.43). Higher levels of sodium lactate (3 and 4%) leaded to increase of vacuoles and more lesions of the lining cells (Fig.44), and dense materials in vacuoles were

exhibited (fig.45). Almost B, R and F-cells are swollen, abnormalities of B-cell were observed; atrophy of the F-cells and of the nucleus; the pyknosis of the hepatopancreatic tubule were seen. Also, connective tissues surrounding the hepatopancreatic tubule were degenerated (Figs.44&45).

The hepatopancreas is a sensitive indicator for metabolism, ecdysis phase, nutritional status, contaminations and diseases in various shrimp species (Al-Mohanna and Nott, 1986; Bautista *et al*., 1994; Rosas *et al*., 1995; Fathallah, 2001; Fernandes-Gimenze *et al*., 2008) and the histology of hepatopancreas supported the trends in growth and feed utilization (Xiao *et al*., 2014). Also, the chief functions of this gland are the secretion of digestive juice into the stomach and absorption of digested food (Miyawaki *et al*., 1984) and store reserve food material in the form of oil globules (Agrawal, 1963). Histological features of the hepatopancreas have been used as a practical means for assessing the nutritional condition in the crustacean culture (Reddy *et al*., 1999; Gimenez *et al*., 2004; Xiao *et al*., 2014).

The present work showed experimentally that, increased level of organic acid salts as acidifiers led to increase of vacuoles, dense materials in vacuoles, swollen of R and B-cells, atrophy of F-cells and more lesion of the cell lining except E-cell. The same results were observed in previous studies by Archanachai (2005) on *Marcobracium lanchesten*, Bray *et al.* (2006) and Li *et al.* (2008) on *L. vannamei*, Fernandes-Gimenze *et al*. (2008) on *Pleoticus muelleri*, Calvo *et al.* (2011) on *Cherax quadricarinatus* and Xiao *et al*. (2014) on *Procambarus clarkii* . Similar observations were reported by other authors such as Vogt *et al.* (1985) and Anderson and Baatrup, (1988) who worked on different decapods. They also claimed that, R and B-cells were sensitive to different diets but R-cell reacts more than other cell types in hepatopancreas this is in agreement with the present work, may due to the vacuoles in the R-cells contain acid glycoconjugates (Nunes *et al*., 2014). The present findings

may be explained by the fact that the maliformation of R and B-cells in hepatopancreatic tissue may due to immune response of the cells (Pinho *et al*., 2003).

The function of the E-cells appears to be replacement of worn out cell, this replacement is not by a directly differentiation. The E-cells undergo mitosis to produce more cells (Davis and Burnett, 1964). In this work, the E-cells of prawn fed on different acidifiers and levels remain relatively intact. Similar results were reported by other authors such as Storch *et al*. (1984), Vogt *et al*. (1985) and Archanachai (2005).

The swelling of B-cells may be related to the synthesis of digestive enzymes function (Al-Mohanna and Nott, 1986; Caceci *et al*., 1988) or due to disorder of neutral polysaccharides present near to the microvilli (Nunes *et al*., 2014). The increased of vacuoles and swelling is due to increased number of R-cell vacuoles, because it is considered the main site for nutrient absorbing that reserve in the hepatopancreas (Al-Mohanna and Nott, 1986; Bray *et al*., 2006) and involves in homeostatic processor that maintain calcium balance (Zilli *et al*., 2003).

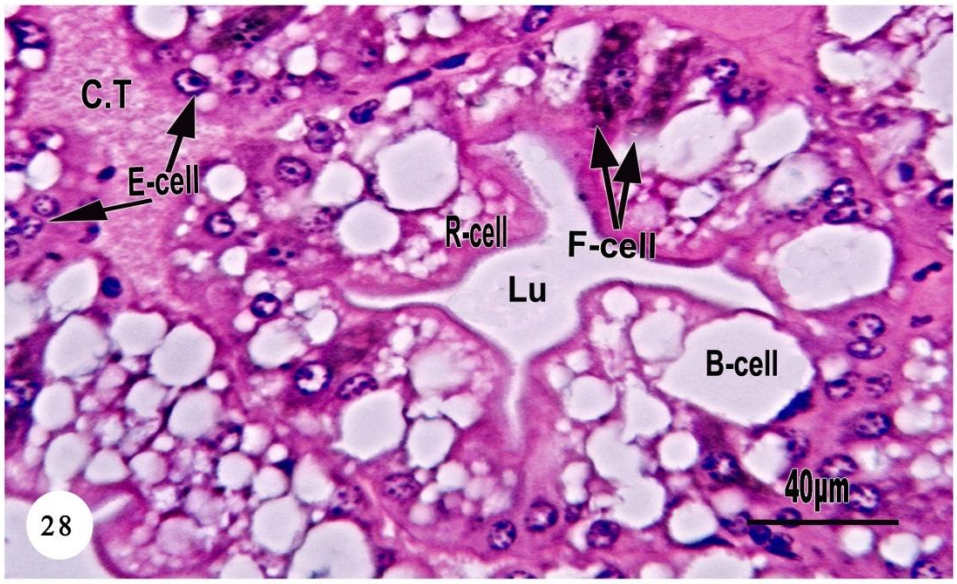
Atrophy of the F-cells and formation of lipid vacuoles inside the F-cells were indication of transformation to R-cells. The transformation of the F-cells to R-cells indicates that the F-cells may take up the toxicant from hemolymph (Archanachai, 2005). Therefore, the present results showed increase of R-cells. The same results were observed in Pacific white shrimp juvenile, *Penaeus vannamei* (Lightner *et al*., 1995) and in riceland shrimp, *Marcobracium lanchesten* (Archanachai, 2005), which showed atrophy in F-cells.

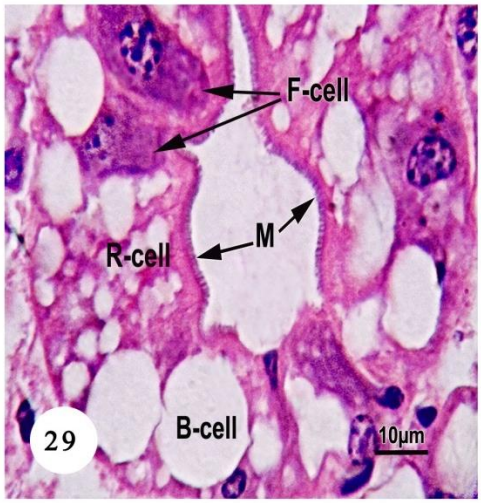
Fig.28: Photomicrograph of a section of the hepatopancreas of a control freshwater prawn *M. rosenbergii* showing the three types of lining cells (B, R and F-cell), (H&E).

Fig.29: Photomicrograph of a section of the hepatopancreas of a control freshwater prawn *M. rosenbergii* showing an apical microvillar border of all type cells, (H&E).

Fig.30: Photomicrograph of a section of the hepatopancreas of a control freshwater prawn *M. rosenbergii* showing the plasma membrane invaginations of B-cell (arrow), (H&E).

B-cell= blasenzellen cell, R-cell= restzellen cell, F-cell= fibrillenyellen or fibrous cell, E-cell= embryonalzellen or embryonic cell, M= microvillar border, Lu= lumen, C.T= connective tissue.


C.T
E-cell
R-cell
F-cell
Lu
B-cell
40µm
28


F-cell
M
R-cell
B-cell
10µm
29

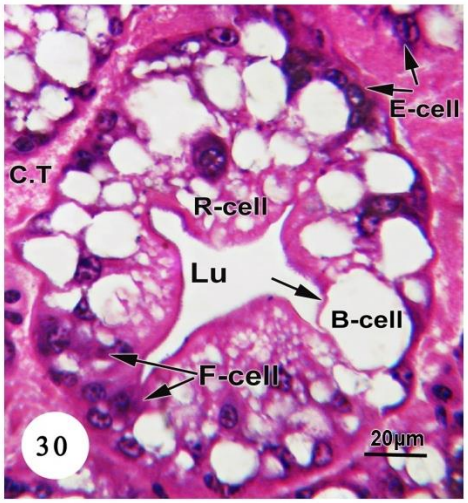

E-cell
C.T
R-cell
Lu
B-cell
F-cell
20µm
30

Fig.31: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 2% sodium lactate showing abnormality of the B and R-cells and atrophy of F-cells, (H&E).

Fig.32: Photomicrograph of a section of the hepatopancreas of a treated freshwater prawn *M. rosenbergii* treated with 3% sodium lactate showing swollen of F-cell, crowding of the vacuoles and nuclei of B and R-cells and few sloughs, (H&E).

Fig.33: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 4% sodium lactate showing hypertrophy and vacuoles of R-cells, degeneration of the F-cells, narrow lumen and some sloughing in the lumen, (H&E).

Fig.34: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 2% calcium lactate showing degenerationof R and B-cells and a lot of slough into the lumen, (H&E).

B-cell= blasenzellen cell, R-cell= restzellen cell, F-cell= fibrillenyellen or fibrous cell, E-cell= embryonalzellen or embryonic cell, Lu= lumen, S= slough, C.T= connective tissue, S= slough.

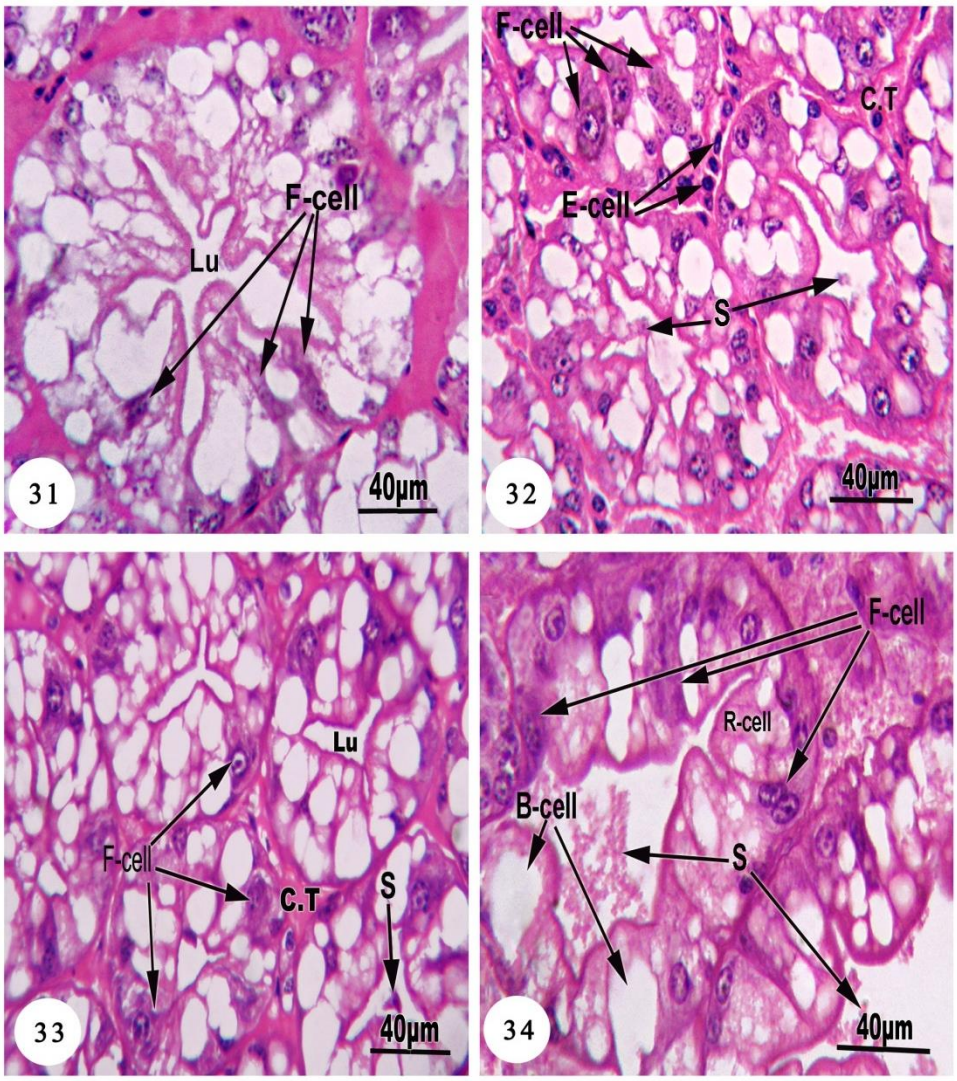

F-cell
Lu
40µm
31
F-cell
C.T
E-cell
S
40µm
32
Lu
F-cell
C.T
S
40µm
33
F-cell
R-cell
B-cell
S
40µm
34

Fig.35: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 3% calcium lactate showing malformation of B, Rand F-cells, (H&E).

C.T= connective tissue, Lu= lumen.

Fig.36: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 4% calcium lactate showing disintegration of B, R and F-cells and some dense materials in vacuoles (arrow), (H&E).

Fig.37: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 2% calcium propionate showing slight destruction of B and R-cells, (H&E).

Fig.38: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 3% calcium propionate showing reduction of lipid droplets storage and few sloughs into the lumen, (H&E).

B-cell= blasenzellen cell, R-cell= restzellen cell, F-cell= fibrillenyellen or fibrous cell, C. T= connective tissue, Lu= lumen, S= slough.

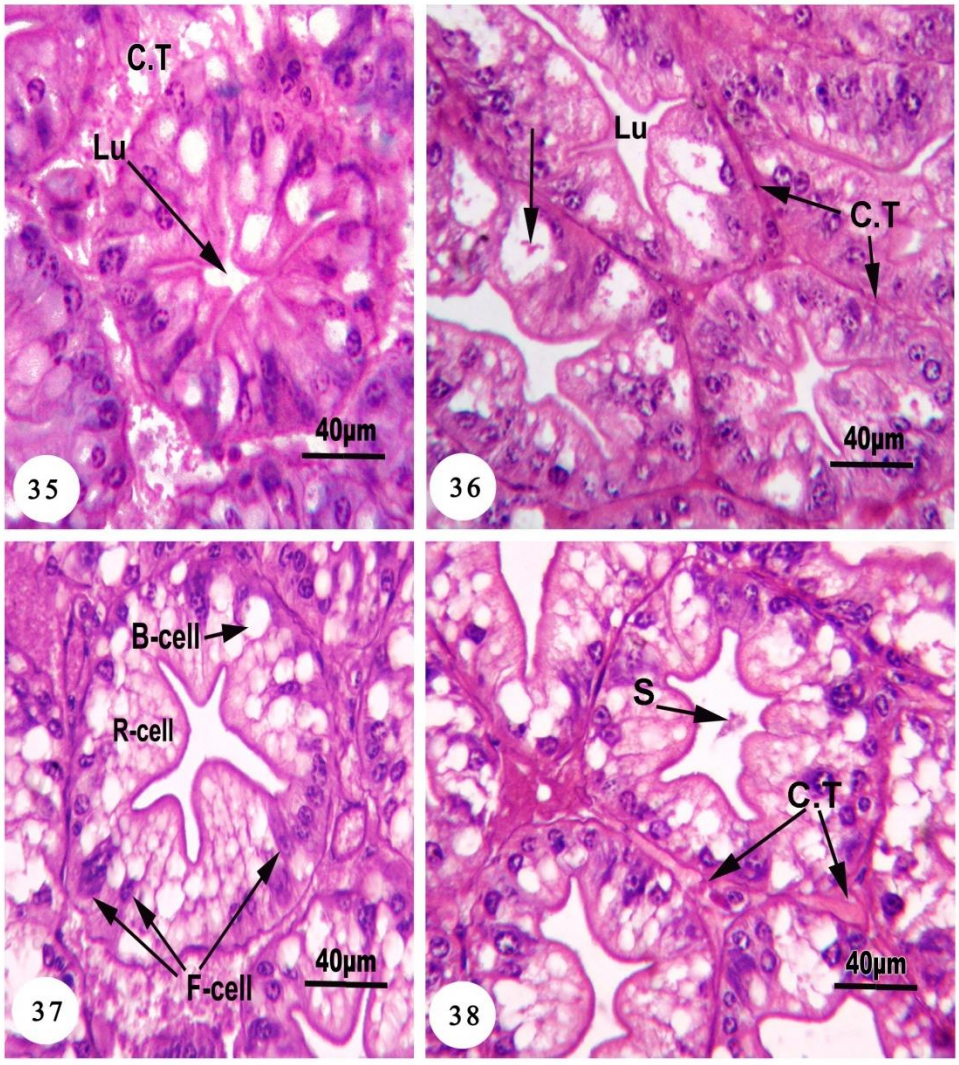

C.T
Lu
40µm
35
Lu
C.T
40µm
36
B-cell
R-cell
F-cell
40µm
37
S
C.T
40µm
38

Fig.39: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 4% calcium propionate showing enlarged vacuoles, slight changing of F-cells shape and narrowing of the lumen, (H&E).

Fig.40: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 1% sodium lactate showing destruction B-cells, a lot of slough in the lumen and enlarged tubular lumen of the distal portion, (H&E).

Fig.41: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 1% sodium lactate showing reduced of lipid vacuoles and narrow lumen of the tubules of the distal portion, (H&E).

Fig.42: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 1.5% sodium lactate showing destruction of the B, R and F-cells of acini and narrow lumen, (H&E).

B-cell= blasenzellen cell, R-cell= restzellen cell, F-cell= fibrillenyellen or fibrous cell, C. T= connective tissue, Lu= lumen, S= slough.

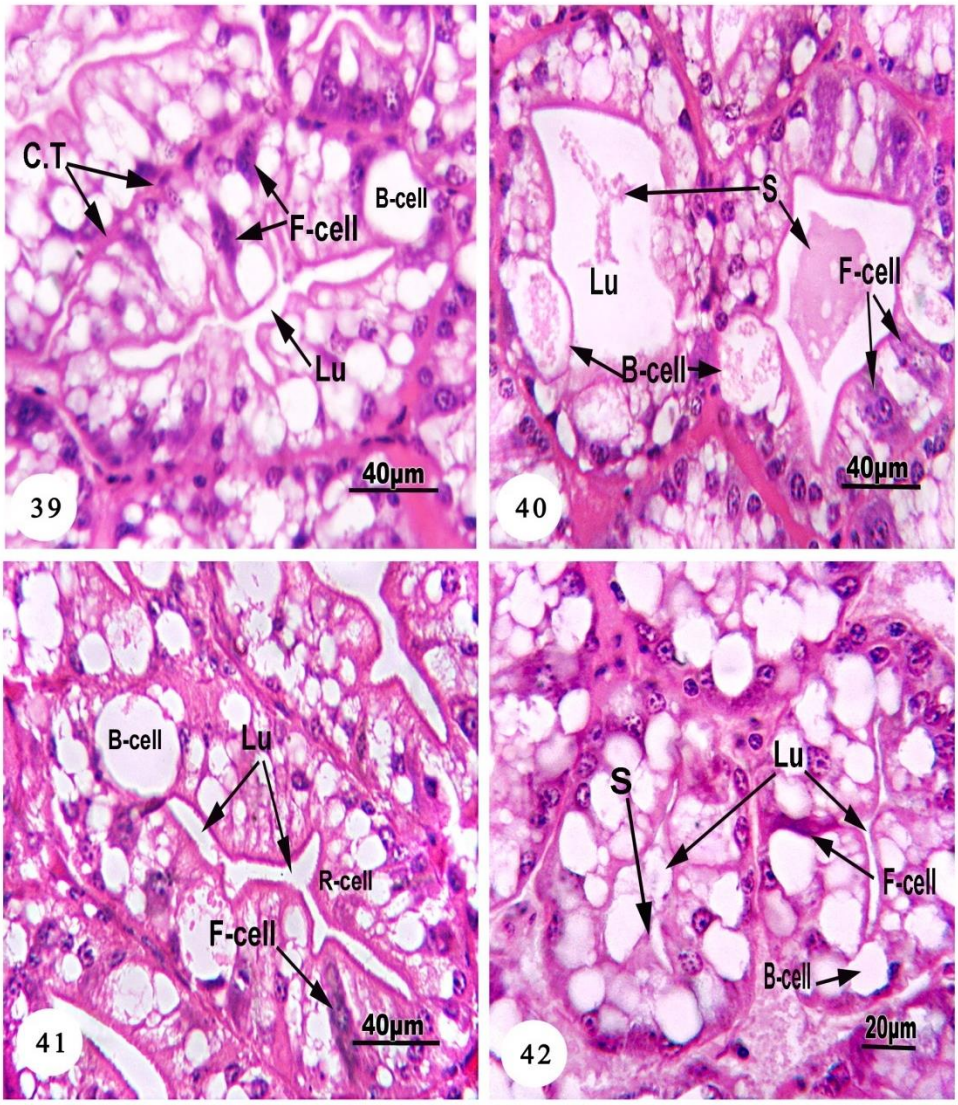
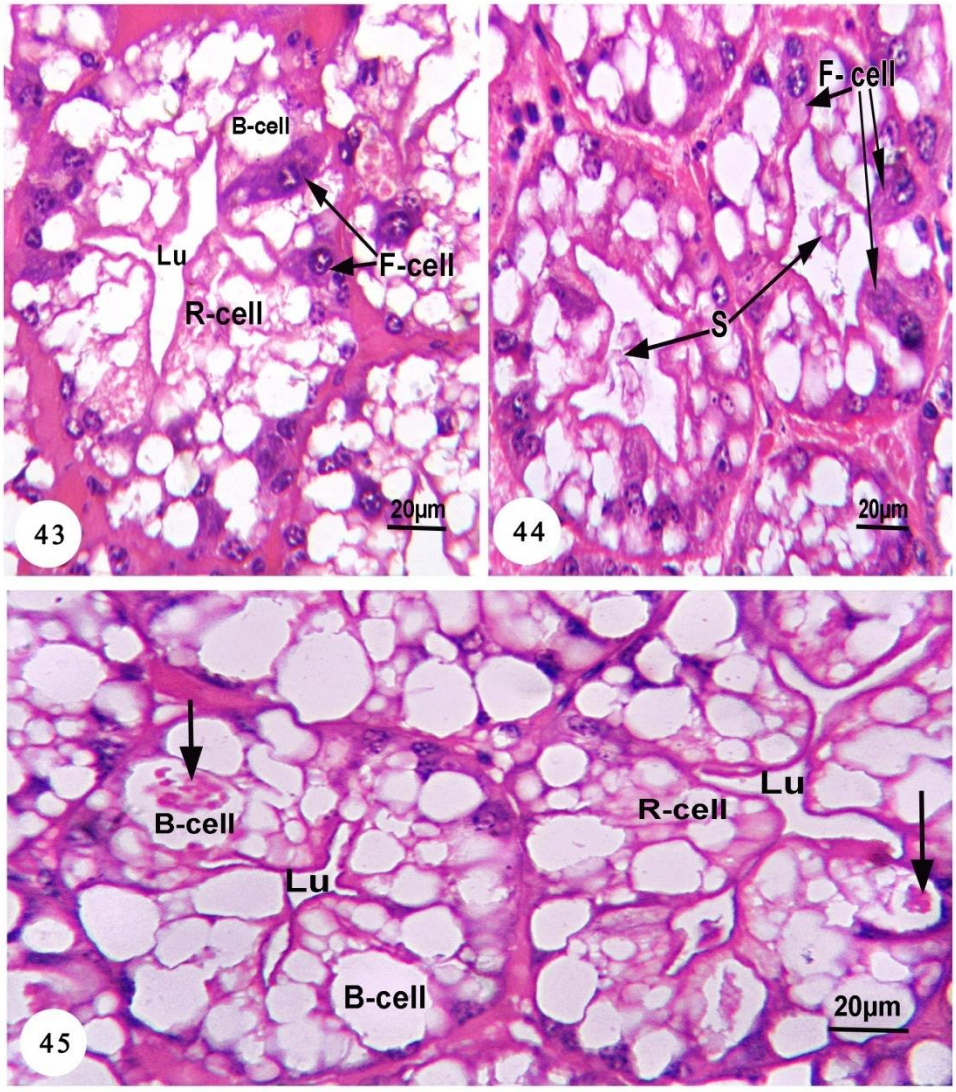

C.T
B-cell
F-cell
Lu
40µm
39
S
Lu
F-cell
B-cell
40µm
40
B-cell
Lu
R-cell
F-cell
40µm
41
S
Lu
F-cell
B-cell
20µm
42

Fig.43: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 2% sodium lactate showing damage of B, R, F-cells and connective tissue, (H&E).

Fig.44: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 3% sodium lactate showing increased vacuoles of R-cells, atrophy of the F-cells and sloughs in the lumen, (H&E).

Fig.45: Photomicrograph of a section of the hepatopancreas of freshwater prawn *M. rosenbergii* treated with 4% sodium lactate showing pyknosis of B, R and F-cells and dense materials in vacuoles of B-cells (arrows), (H&E).

B-cell= blasenzellen cell, R-cell= restzellen cell, F-cell= fibrillenyellen or fibrous cell, Lu= lumen, S= slough.


B-cell
Lu
F-cell
R-cell
20µm
43
F- cell
S
20µm
44
B-cell
Lu
R-cell
Lu
B-cell
20µm
45

3.2.2.2. Gonads

3.2.2.2.1. Control prawn (Group I)

3.2.2.2.1.1. Ovary

The ovary of the mature giant freshwater prawn *M. rosenbergii* has two lobes situated underneath the carapace dorsal to the hepatopancreas and ventral to the heart. It is color (transparence, bright yellow, and orange) depending on the ovarian development which gradually changes. The ovary supports by hemolymph into vessels called hemolymph sinus.

Histologically, the developing ovary is invested by ovarian capsule made of fibromuscular tissue. Parts of the capsule extended as sheet-like structure into the interior of the ovary. These structures divide the ovarian tissue of each lobe into cone-shaped ovarian pouches or lobules, each containing various steps of oocytes according to the stages of ovarian development. Based on the number and steps of differentiating oocytes the growing ovarian pouches can be divided into four zones: oogenic (Oz), previtellogenic (Pz), vitellogenic (Vz) and mature zones (Mz) (Fig.46). The oogenic zone (Oz) is the inner most region facing the central ovarian core. This zone contains dividing oogonia (Oog) and primary oocytes (pOc), which predominate in ovarian stage I. The previtellogenic zone (Pz) is located next to the oogenic zone and contains previtellogenic secondary oocytes (Oc1 and Oc2). This zone predominates in the ovarian stage II. The vitellogenic zone is located in peripheral area of the ovary and contains vitellogenic secondary oocytes (Oc3 and Oc4). This zone predominates in ovarian stage III. In ovarian stage IV, the maturation zone contains fully mature oocytes (mOc) which are distinguished by deep acidophilic (reddish) stain, and the zone expands throughout the ovarian tissue of mature ovary.

The oogonia (Oog) are an ovoid cell that contains an irregularly shaped nucleus with deep blue. Oog are frequently seen in groups, each with 2–4 cells in a row at the tip of the ovarian pouch (Fig.47). Primary oocytes (pOc) accumulate at the oogenic zone, and are characterized by the nuclei containing dense small cords of heterochromatin (Fig.47); therefore, pOc appear in various steps of 1st meiosis. Their sizes are slightly larger than the size of Oog.

Secondary oocytes phase of developing oocytes is undergoing 2nd meiosis and they are classified into five steps, which include Oc1, Oc2, Oc3, Oc4, and mOc. Early previtellogenic oocyte (Oc1) is characterized by a substantial increase in size (ranging from 10–30 μm in diameter) when compared to the primary oocytes (Fig.47). The

nucleus is characterized by the presence of the intensely blue-stained, and the rest of the nucleoplasm appears very light. The nucleolus is small and not always clearly visible. Oc1 occupy a zone external to the Oog and pOc. Late previtellogenic oocyte (Oc2) is characterized by the increase in cell size. The cytoplasm is enlarged and exhibits more intensely blue staining (Fig.47). The nucleus is characterized by the presence of dense blocks of heterochromatin scattering throughout. The centrally located nucleolus becomes prominent with densely packed materials. Early vitellogenic oocyte (Oc3) is characterized by the decrease of bluish stainand the increase of reddish stain in the cytoplasm (Fig.47). The cytoplasm also contains some lipid droplets, which are distributed randomly at the periphery. The round nucleus is enlarged and still centrally located. Late vitellogenic oocyte (Oc4) displays violet-stained acidophilic cytoplasm with increasing amount of lipid droplets and reddish proteinaceous yolk plaques (Py). At this stage of oocyte, the lipid droplets apparently increase in size and become located along the cytoplasmic rim (Fig.46).

Mature oocyte (mOc) is characterized by a remarkable increase in cell size, more than ten times the early previtellogenic oocytes, and has a hexagonal shape (Figs.46&48). The cytoplasm becomes highly acidophilic and filled with large lipid droplets and proteinaceous yolk plaques. The nuclear membrane is not apparent and frequently disappears.

3.2.2.2.1.2. Testis

The testis of *M. rosenbergii* lies dorsally in the thorax between the hepatopancreas and the heart. It consists of elongated paired anterior lobes and posterior lobes. The posterior lobes lie beneath the heart and are smaller than the anterior lobes.

Histologically, it is composed of several seminiferous tubules (St) grouped together into lobules, each of which is surrounded by a thin layer of connective tissue. The spermatogonia are situated at the periphery of the seminiferous tubule, while several types of spermatocytes are located towards the center of the tubule (Fig.49). Part of the epithelium was multilayered and included cells of variable size and forming a spermatogenic zone (Sz); while the mature sperm (Sp) are found in the lumen of tubules which are connected with the proximal part of the vas deferens. In addition, nurse cells (Nc) are observed on the basement membrane between the spermatogonia (Fig.49).

Spermatogenesis of *M. rosenbergii* testis, were exhibited in figures (49) which including spermatogonia, spermatocytes, spermatids, and sperms or spermatozoa.

Spermatogonia are located at the periphery of the seminiferous tubules. A spermatogonium (Sg) is an oval shaped cell, with a large centrally located nucleus, and a thin cytoplasmic rim. Nurse cells are also located at the periphery of the tubule in close association with the spermatogonia. Spermatocytes (Sc) are spherical cells with more size than spermatogonia. Its chromatin exhibits highly condensed cord-like structures that are aggregated together.

In the spermatids, most condensed chromatin appears as a small crescentic structure confined to one side of the nucleus. Spermatozoa (Sp) consists of a dense anterior part, comprising a spike-like projection and its base plate, thereby resembling an everted umbrella, and a posterior part which appears globular and clear (Figs.49).

In the present results, the oogenesis and spermatogensis of *M. rosenbergii* are similar with other studies on different decapod crustaceans (Sarojini *et al*, 1986; Yano and Hoshino, 2006; Marsden *et al*., 2007; Kruevaisayawan *et al*., 2010; Saad and Hassan, 2010; Ferreira *et al*., 2012). On the other hand, many authors described oogenesis and spermatogensis of *M. rosenbergii* in details (Lynn and Clark, 1983; Dougherty and Sandifer, 1984; Sagi *et al*., 1988; Lee and Chang, 1997; Meeratana and Sobhon, 2007; Poljaroen *et al*., 2010; Rungsin *et al*., 2012).

3.2.2.2.2. Treated prawn

3.2.2.2.2.1. Experiment II (Group II)

Results of freshwater prawn *M. rosenbergii* treated with different levels (2, 3 and 4%) of three kinds of organic acid salts as acidifiers (sodium lactate, calcium lactate, and calcium propionate). After 74 days showed that no destructions were detected in the gonads. Sections of the ovary and testis exhibited its normal pattern showing normal oocytes and spermatocytes.

3.2.2.2.2.2. Experiment II (Group III)

The gonads of freshwater prawn *M. rosenbergii* treated with sodium lactate (1, 1.5, 2, 3 and 4%) after 103 days did not reveal any histological alternations of the ovary and testis.

The studies about the effects of the feed additives on the gonad of decapods were very rarely carried out. The present results are agreement with Bray *et al.* (2006), who added Oxytetracyclin (antibacterial) to diet of *Litopenaeus vannamei* and observed no lesions of histological features in gonad. While, Sarojini *et al*. (1986) revealed drastic changes in the larvae and gonads of *Machrobracium lamerrii*, exposed to

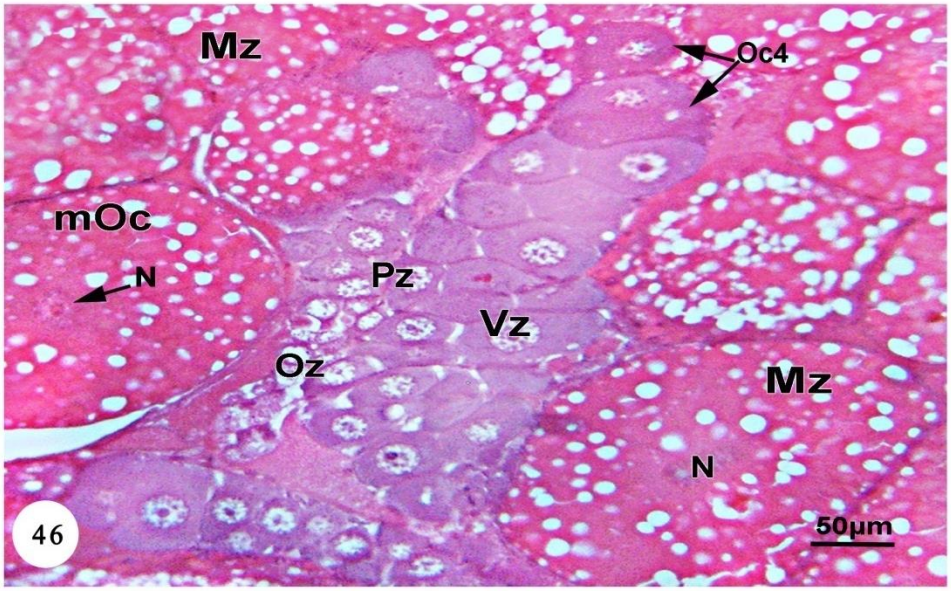
fenitrothion (insecticide). Also, Aly (2000) noticed that deformation and central haemorrhage of oocytes after exposed of *Procambrus clarkii* from river Nile to insecticides. Meeratana *et al*. (2006) suggested that serotonin indirectly induces ovarian development and oocytes maturation in *M. rosenbergii*. Moreover, Revathi *et al*. (2011) investigated the effect of cadmium chloride on oogenesis of freshwater prawn, *Macrobrachium rosenbergii* and showed reduction of yolk material and thickness of oocyte membrane.

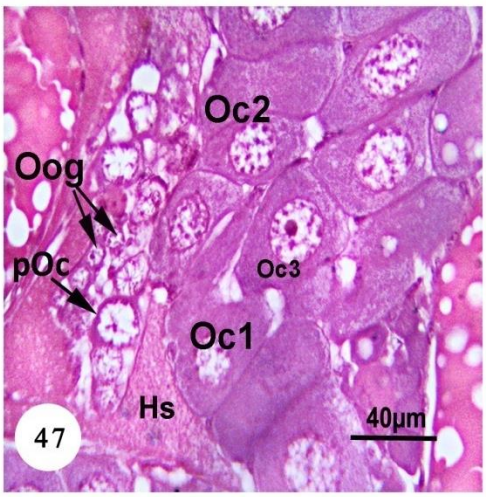
Fig.46: Photomicrograph of a T.S. of the ovary of a control freshwater prawn *M. rosenbergii* showing four ovarian zones, (H&E).

Fig.47: Photomicrograph of a T.S. of the ovary of a control freshwater prawn *M. rosenbergii* showing various stages of developing germ cells and oocytes, (H&E).

Fig.48: Photomicrographs of T.S. of the ovary of a control freshwater prawn *M. rosenbergii* showing mature oocyte at mature zone, (H&E).

Oz= oogenic zone, Pz= previtellogenic zone, Vz= vitellogenic zone, Mz= mature zone, N= nucleus, pOc= primary oocytes, Oc1, Oc2 and Oc3= previtellogenic oocytes, Oc4= vitellogenic oocyte, mOc= mature oocyte, Li= lipid droplets, Py= proteinaceous yolk plaques, Hs= hemolymph sinus.


Mz
Oc4
mOc
N
Pz
Vz
Oz
Mz
N
46
50µm


Oc2
Oog
pOc
Oc3
Oc1
Hs
47
40µm

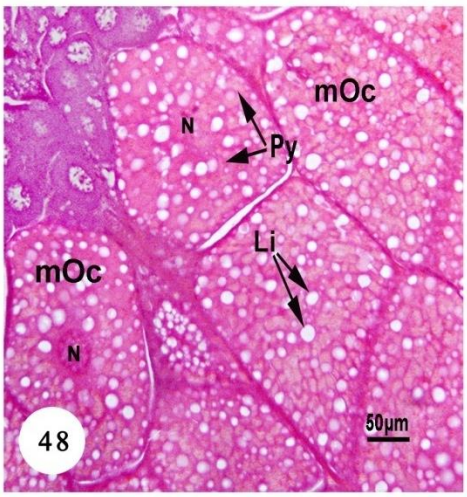
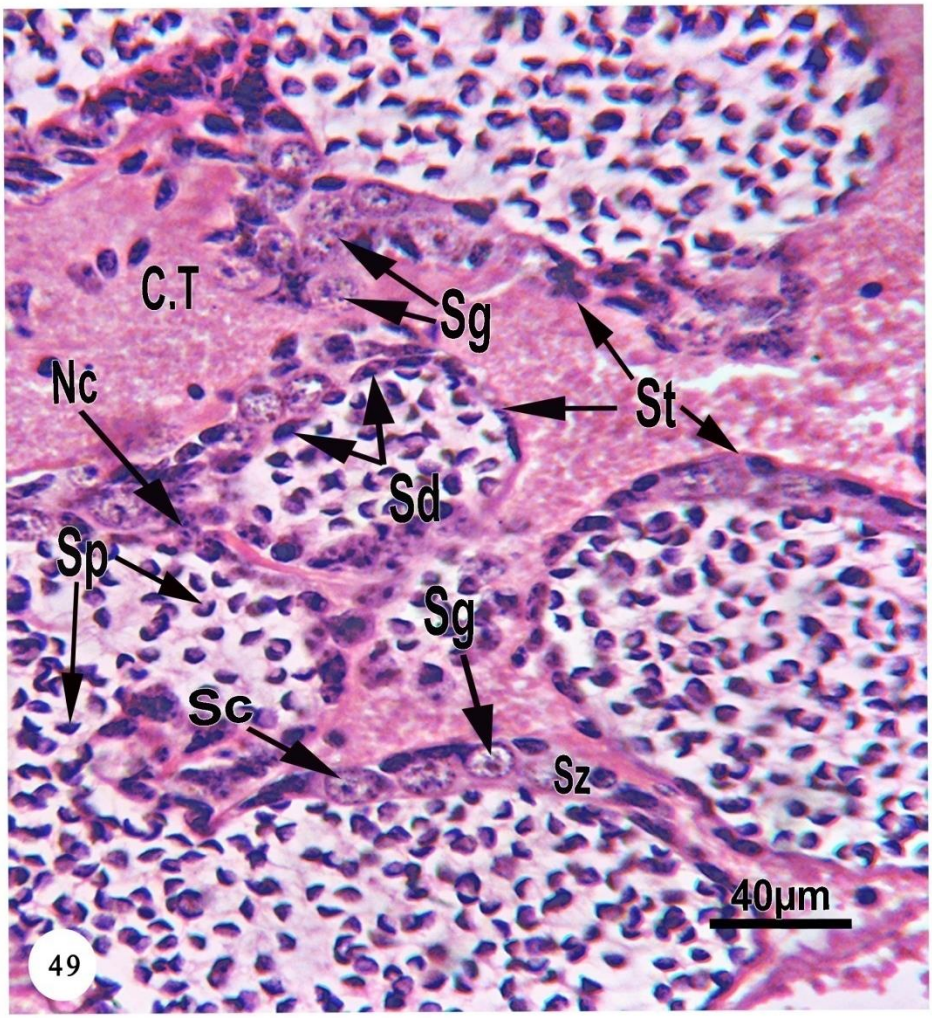

mOc
N
Py
Li
mOc
N
48
50µm

Fig.49: Photomicrograph of a T.S. of the testis of a control freshwater prawn *M. rosenbergii* showing two spermatic zones and seminiferous tubules, (H&E).

St= seminiferous tubules, Sz= spermatogenic zone, Sg= spermatogonia, Sc= spermatocytes, Sd= spermatids, Sp= spermatozoa, Nc= nurse cells, C.T= connective tissue.



4. SUMMARYOF THIS BOOK

1) The higher growth performance of *O. niloticus* under the present experimental conditions was observed at 4% sodium lactate and the calcium propionate improved FCR at all levels.
2) All organic acids salts improved the apparent protein and lipid digestibility of Nile tilapia.
3) The histological work of liver, pancreas and testis of *O. niloticus* exhibited that 2% of all organic acid salts as acidifiers used are more suitable level than 3% and 4%. 2% proved to be significantly more convenient to tilapia than 3% and 4% as growth promoter. The testis tissue revealed that high level (4%) of all types of acidifiers used, cause testicular histopathological effects. Although, 4% sodium lactate recorded higher growth rates. Regarding to ovary, it intact
4) The growth performance of *M. rosenbergii* improved at 2% calcium lactate, 3% calcium propionate and 1% sodium lactate.
5) The apparent protein and lipid digestibility of freshwater prawn improved at 4% sodium lactate.
6) Hepatopancreas tissue showed mild histological effect at 2 and 3% calcium propionate and 1% sodium lactate for *M. rosenbergii*. The gonads of freshwater prawn are intact.

5. RECOMMENDATIONS

- The best organic acid salts as acidifiers of *O. niloticus* is 2% calcium propionate, and of *M. rosenbergii* is 2, 3% calcium propionate and 1% sodium lactate for the best biological results associated with the best histological results exhibited with the low levels.
- This book suggested focusing on the lower level of organic acid as acidifiers (less than 2%), obtain better results.
- Further immunological works are needed to clarify the role of organic acid salts as acidifiers in enhancement of immunity system of Nile tilapia and freshwater prawn.

6. REFERENCES

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7. ABBREVIATIONS

ATP	adenosine triphosphate	Ps	primary spermatocyte
B-cell	blasenzellen cell	Pt	pancreatic tissue
Bv	blood vessel	Pv	portal vein.
C	congestion.	Py	primary yolk stage
C.T	connective tissue	R	rupture tissue
CP	crude protein	Rc	reticulo-endothelial cells
DW	dietary weight	R-cell	restzellencell
E-cell	embryoniccell	S	slough
EpO	early perinucleolusoocyte	S	sinusoid
EU	european Union	Sd	spermatids
F-cell	fibrous cell	Sg	spermatogonia
H	hepatocyte	Ss	secondary spermatocyte
He	hemorrhage	St	seminiferous tubules
LC	leydig cells	Sy	secondary yolk stage
Ld	lipid droplet.	Sz	spermatozoa
LpO	late perinucleolusoocyte	Ta	tunica albuginea
Lu	lumen	Tf	theca folliculi
M	microvillar border	Ti	theca interna
Mt	million tons	Ty	tertiary yolk stage
N	nucleus	V	vacuole
Ne	necrosis	Yg	yolk granule
NRC	national Research Council	Yv	yolk vesicle stage
Og	oogonia	Zr	zonaradiate
Pc	pancreatic acinar cell		
pK	dissociation constant		
PL	post-larvae		