Impact of sodium lactate as a growth promoter on the hepatopancreas of the freshwater prawn *Macrobrachium rosenbergii* (de Man, 1879).

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ABSTRACT

In the present study, histological changes of hepatopancreas were studied in response of using sodium lactate as a growth promoter to the freshwater prawn *Macrobrachium rosenbergii*. The prawns were fed on diet with five concentrations of sodium lactate (1, 1.5, 2, 3 and 4g/kg of diet) as food supplement for 103 days.

Results showed that R and B-cells were very sensitive to different diets. Slight changes were apparent in the hepatopancreas in the 1% concentration. While, at 1.5 % concentrations, swollen of the cells and enlarged of the vacuoles were observed. Regarding the concentrations of 2%, more accumulation of vacuoles was exhibited. Increase of vacuoles in the R-cells, abnormalities of B-cells, atrophy of the F-cells and pyknosis in the hepatopancreatic cells at 3 & 4% were observed.

This investigation and other results of the detailed study of the same authors concluded that the best using concentration of sodium lactate is 1%, because it has mild histological effects and at the same times, it has the best promotion for the growth rate of *M. rosenbergii.*

Keywords: *Macrobrachium rosenbergii*, sodium lactate, organic acids, acidifiers, hepatopancreas.

INTRODUCTION

Since January 2006, the European Union banned the use of antibiotics in animal production, which is a worldwide trend, because they lead to the transfer of resistance to bacterial species pathogenic for fish and shrimps (Lückstädt, 2006; Aftabuddin *et al*., 2009; Budiati *et al.*, 2013).Thus, a great interest has arisen in seeking alternatives to antibiotic substances that could inhibit pathogens and also act as growth promoters (Lim *et al*., 2010). In this context, organic acids or their salts have become a promising alternative feed additive for aquatic animal (Lückstädt, 2008; Ng and Koh, 2011).

Organic acids and their salts can also contribute in nutritional ways, because they are components in several metabolic pathways for energy generation, for instance, for ATP generation in the citric acid cycle or carboxylic-acids cycle. Whereas, diet acidification significantly reduces the pH diet, but does not affect the gastrointestinal pH. The use of acids in diets for pigs enhanced the nutrient digestibility and dissimilarly affected the microbial populations in different parts of the digestive tract (Freitag, 2007; Lückstädt, 2008). It is generally accepted that several short organic acids, including lactate carry an antibacterial potential (Sissons, 1989). Lactic acid is normally formed as major metabolite from pyruvate in muscles of higher organisms when the supply of oxygen is limited; for example, during intense muscle activity. Accordingly, it has been regarded as a by-product of little importance in fish nutrition (Gislason *et al*., 1996). The effect of lactic acid is probably related to environmental pH. At or below its pH value of 3.9, lactic acid is mainly present in its

nonpolar undissociated form. Because of the low polarity, it can readily diffuse across bacterial cell membrane (Hinton, 1990).

In the first studies with acidifiers in the aquaculture, Ringo (1991) and Ringo *et al.* (1994) were observed that the lactate supplementation of 1% in the feed of Arctic charr, *Salvelinus alpines* increased growth and feed efficiency. It also decreased the incidence of diarrhea during cultivation. Liebert *et al.* (2010) showed that when 0.3% of sodium diformate was added in the diet of the tilapia fingerlings; *Oreochromis niloticus*, the protein efficiency ratio and protein retention significantly improved. However, studies on marine shrimps fed on organic acids or their salts are limited.

Lückstädt (2008) reported the highest growth of the shrimp *Marsurpenaeus japonicus* during the addition of 0.5% sodium citrate with inactivated lactobacilli.

Nuez-Ortin (2011) suggested that 0.25% calcium formate improved *Penaeus monodon* survival in Taiwan farms. Anuta *et al.* (2011) found that the addition of 0.4–2% commercial acid, based on calcium sulfate, did not change the performance parameters of the marine shrimp, *Litopenaeus vannamei*. However, they noted an increase in the immune response and a change in the intestinal microbiota. *Penaeus monodon* supplemented with 1% of commercial acidifiers with sodium butyrate enhanced the digestibility of the dry matter, crude protein and energy, leading to numerical improvements on weight gain, survival and feed conversion ratio (Nuez-Ortin, 2011). *L. vannamei* shrimp supplemented with 0.5% of potassium diformate showed an increase of 19% on productivity in comparison to non-supplemented shrimp (Kühlmann *et al*., 2011).

On the other hand, the hepatopancreas is the major digestive gland and also is a sensitive indicator for metabolism, ecdysis phase, nutritional status, and diseases in various shrimp species (Bautista *et al.*, 1994; Rosas *et al*., 1995). Histological studies on the hepatopancreas have been used as a practical means for assessing the nutritional condition in the shrimp culture (Reddy *et al*., 1999; Gimenez *et al*., 2004).

The objective of this study is to examine the changes of histological structure of hepatopancreas after treatment by three concentrations of sodium lactate as feed additive for the freshwater prawn *Macrobrachium rosenbergii* in Egypt.

MATERIALS AND METHODS

This experiment was conducted from August 22th, 2009 to December 2nd, 2009 (103 days). One thousand and eight hundred freshwater prawns, *M. rosenbergii* PL were divided into eighteen groups and stocked into eighteen cement pens (0.96 m3) at Fish Research Station, El-Kanater El-Khayria, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt. Triplicate pens were randomly assigned to each treatment. All pens were supplied with freshwater prawns from the Darawa Irrigation Branch, Kalubiya Governorate where the water turnover rate was 0.25 m^3 pen twice weekly. 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).

During the experimental period, photoperiod was held under natural light (12:12 h light: dark schedule). The feeding rate begins from 20% and decreased 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.

Six 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, 4, 5 and 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 diets is presented in Table (1).

Randomly 4 specimens from each replicate of *Macrobrachium rosenbergii* were chosen and dissected. The hepatopancreas were fixed in Bouin's fluid. 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 haematoxylin counterstained with eosin and mounted in DPX (Yano, 1988). 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 1. Ingredients and proximate composition (%) of the experimental tested diets used in the experiment.

Item	Diet					
Ingredients(g|kg)	1	2	3	4	5	6
Fish meal	340	340	340	340	340	340
Soybean meal	270	270	270	270	270	270
Wheat bran	250	250	250	250	250	250
Yellow corn	60	60	60	60	60	60
Soybean oil	20	20	20	20	20	20
Linseed oil	20	20	20	20	20	20
Vits and minerals	40	40	40	40	40	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 Acidifiers); diets 2,3,4,5, and 6 were containing 1, 1.5, 2,3and4 % sodium lactate. Gross energy (GE2) 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). P/E ratio3 = protein energy ratio.

RESULTS AND DISCUSSIONS

Control shrimp

The hepatopancreas (mid gut gland or digestive gland) of the normal *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 a light beige color. These two lobes are not separated from each other and 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. This agrees with Vogt *et al*. (1985) who studied the midgut gland of the *Penaeus monodon*. Several conflicting reports are found in the literature. Vogt *et al*. (1994); Archanachai (2005); Bray *et al*. (2006); Calvo *et al*. (2011) reported that the hepatopancreas of different decapods divided into three parts (proximal, middle, and distal). This subdivision depends on the shape of hepatopancreatic gland.

The gland is composed of a great number of oval or circular acini (tubules). Each acinus is lined with simple epithelial cells and separated from the neighboring ones 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 the tubule are arranged around a wide irregular lumen. The cross section of the lumen has a star-like shape (Fig.1a). The lining epithelium of the tubules is composed of four main types of cells. Embryonic or embryonalzellen cell ``E-cell''; its shape varies between cuboidal and columnar epithelial cells, with rounded to oval nuclei, each containing conspicuous nucleolus. The nucleus of E-cells is in the middle region of the cytoplasm and predominates in distal tubule tips (Fig.1b). Restzellen cell ``R-cell`` is columnar in shape, has a basal rounded nucleus, and bears an apical microvillar border (Fig.1a); it contains variable sized small lipid vacuoles. Lipid droplets have been found only in R-cells (Fig.1c). Blasenzellen cell ``B-cell'' contains a giant single large secretary vesicle in the center of the cell and occupying most of the cell size. Plasma membrane invagination in some of the B-cells was also observed, this may be due to absorption of materials from the lumen (Fig.1a). The nucleus of B-cell is lateral and flat to oval in shape. Fibrillenyellen or fibrous ``F-cell'' is spindle in 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 eosin (Figs.1a & c). 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.

This is in agreement with similar studies on other decapod crustaceans (Storch *et al*., 1984; Vogt *et al*., 1985; Aly, 2000; Sayed, 2002; Bray *et al*., 2006; Li *et al*., 2008; Leser *et al*., 2008 and Calvo *et al*., 2011). A more or less similar results were given by Aly (2000); Sayed (2002) who worked on the crayfish (*Procambarus clarkii*). They suggested the presence of two main types of cells depending on the function, digestive and secretory cells. The digestive cells included R and B-cell, and the secretory cell is F-cell.

Treated shrimps

In the present work, the hepatopancreas of the giant prawn *M. rosenbergi* reared in five concentrations of sodium lactate (1, 1.5, 2, 3 and 4g/kg of diet), were studied.

In the treatment of 1% sodium lactate, for 103 days, the hepatopancreas revealed little degeneration of the large secretary vesicle of B-cell leading to presence of dense materials in vacuoles; also, reduction of lipid vacuoles of R-cell can be observed (Figs. 2a, b & c). The lumen of the tubules has some slough as a result of damage of the epithelial cells of tubules (tissue debris). This led to an enlargement of tubular lumen in the distal portion, while the proximal portion became narrow (Fig. 2a). The connective tissues surrounding of hepatopancreas tubule were slightly degenerated and shrinked (Figs. 2a & c). Clearly, the destruction of hepatopancreas is increased in the proximal than that of the distal portion due to the proximal one has more number of R-cells and B-cells, and it is close to midgut.

At the 1.5% sodium lactate treatment, it was found some changes in the shape of all cell types except E-cell (Fig. 3a). Very narrow lumen due to swollen of the cells, increased and enlargement of the vacuoles were observed (Fig. 3b).

Regarding the 2% sodium lactate treatment, more accumulation of vacuoles, damage of hepatopancreatic tubules and connective tissues were observed (Fig. 4a). Higher concentrations of sodium lactate (3 and 4%) led to increase of vacuoles, more lesion of the cell lining (Fig. 4b), and dense materials in the vacuoles were exhibited (Fig. 4c). Almost all of the cells in hepatopancreatic tubules in these concentrations observed swollen, increased abnormalities of B-cells, atrophy of the F-cells and of the nuclei; pyknosis of the hepatopancreatic tubules. Also, connective tissues surrounding the hepatopancreatic tubules were degenerated (Figs. 4b & c).

The present work showed experimentally that, increased concentration of sodium lactate led to increase of vacuoles, dense materials in vacuoles, swollen of R and B cells and more lesion of the cell lining, except E-cell. This is in agreement with Archanachai (2005) on *Marcobracium lanchesten*, Bray *et al.* (2006) and Li *et al*. (2008) on *L. vannamei* and Calvo *et al.* (2011) on *Cherax quadricarinatus*. The hepatopancreas serves to secrete digestive enzymes and to store reserve food material in the form of oil globules and thus subserve the functions of the liver and pancreas in chordates (Agrawal, 1963).

Vogt *et al.* (1985), Archanachai (2005) and Bray *et al.* (2006) studied different decapods and stated that, R and B cells were sensitive to different diets but R-cell reacts more than other cell types in hepatopancreas and this is in agreement with the present work. The swelling of B-cells may be related to the synthesis of digestive enzymes function (Al-Mohanna and Nott, 1986; Caceci *et al*., 1988). 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, 1989; Bray *et al.*, 2006) and involves in homeostatic processor that maintain calcium balance (Zilli *et al*., 2003).

In a detailed study for the authors (unpublished data), it was found that 1% is the ideal concentration for growth performance of *M. rosenbergii.* Therefore, we can say that the best concentration of sodium lactate is 1%, because it has mild histological effects and at the same times, it has the best promotion for the growth rate of *M. rosenbergii.*

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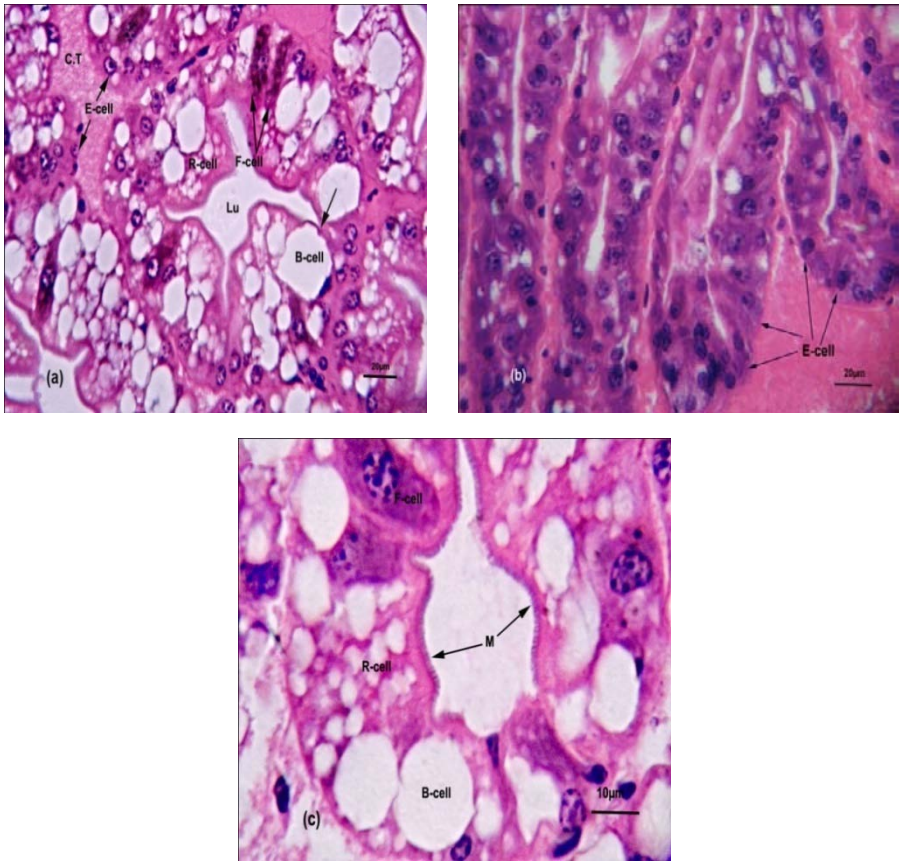

Fig. 1: Photomicrographs of hepatopancreas sections of a normal prawn *M. rosenbergii*, showing (a): three types of lining cells (B, R and F-cell) and the plasma membrane invagination of B-cell (arrow), (b): E-cell in distal tubule tips, (c): an apical microvillar border of all type cells, (H&E).
E-cell: Embryonic or embryonalzellen cell, R-cell: Restzellen cell, B-cell: Blasenzellen cell, F-cell: Fibrillenyellen or fibrous cell, M: microvillar border, Lu: lumen, C.T: connective tissue.

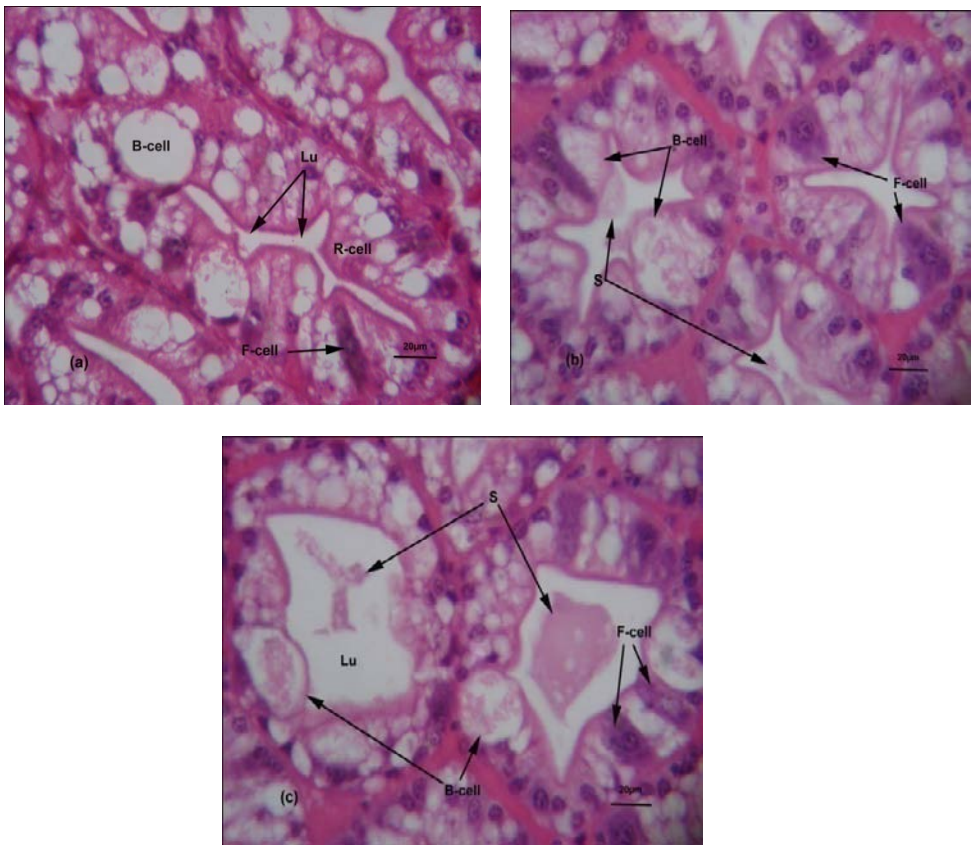

Fig. 2: Photomicrographs of hepatopancreas sections of the prawn *M. rosenbergii* treated by 1% sodium lactate showing, (a): narrow of the lumen of the distal portion, (b): deformation of B-cell and reduced of lipid vacuoles, (c): deformation of R, B and F-cells and a lot of slough (S) in the lumen (Lu), (H&E).

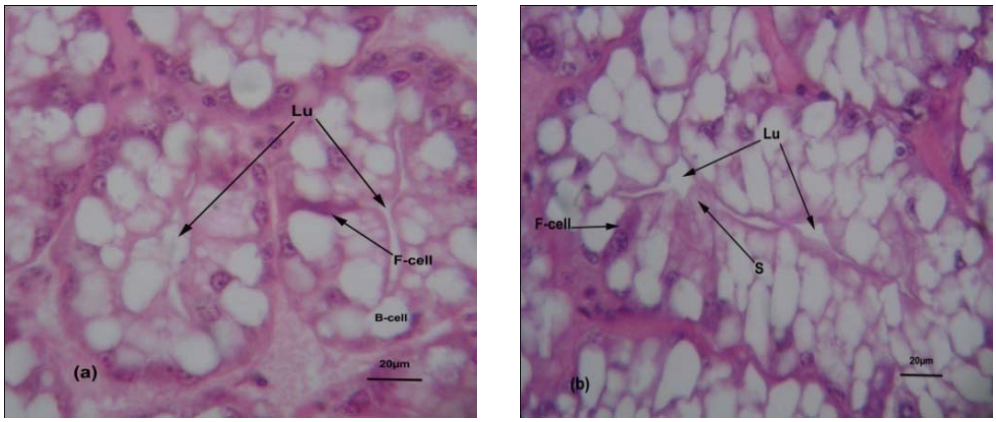

Fig. 3: Photomicrographs of hepatopancreas sections of a treated prawn with 1.5% sodium lactate showing (a): deformation of the lining cells of acini, (b): narrow lumen (Lu), increase vacuoles and slough (S) (H&E).

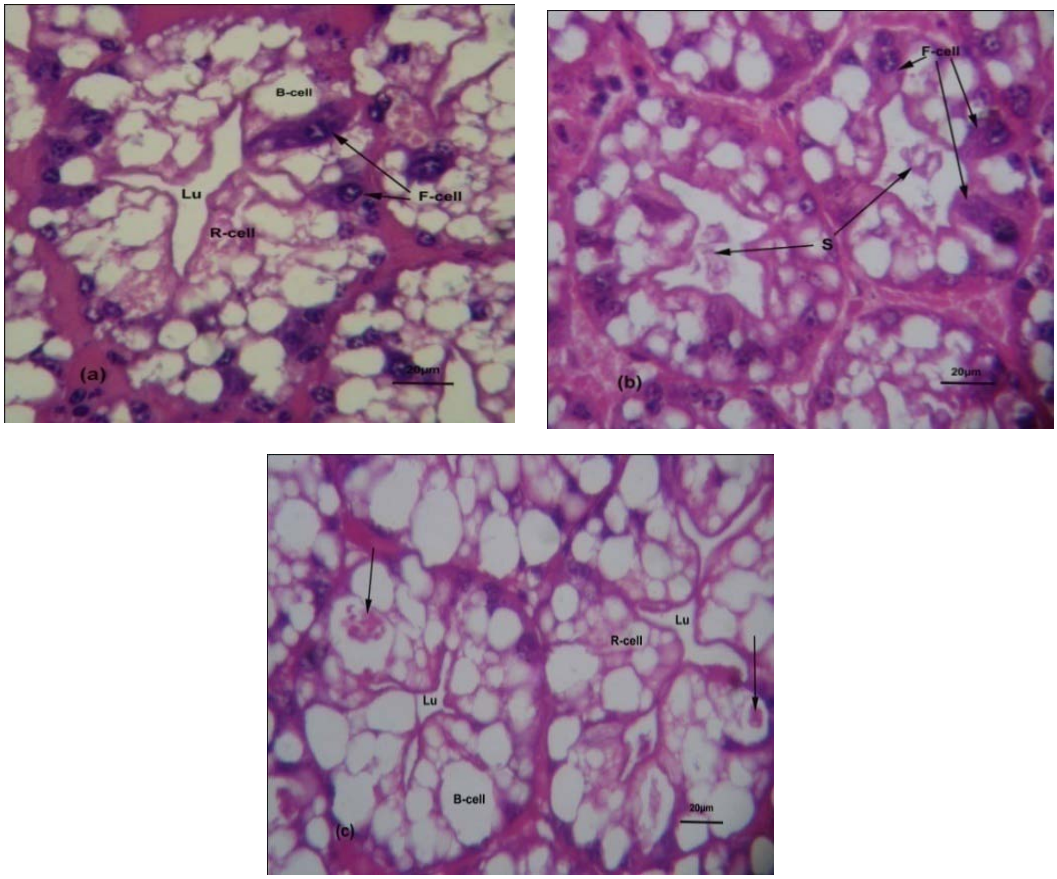

Fig. 4: Photomicrographs of hepatopancreas sections of a treated prawn with sodium lactate at (a) 2%, (b) 3%, showing damage of hepatopancreatic tubules and connective tissue. (c): 4% showing dense materials in the vacuoles (arrows), (H&E).

ARABIC SUMMRY

تأثير لاكتات الصوديوم كمحفز للنمو على الكبد- بنكرياس لجمبري المياه العذبة ماكروبريكيم روزنبيرجي .

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٢- المعهد القومي لعلوم البحار والمصايد - القاهرة - جمهورية مصر العربية.

أجريت هذه الدراسة لمعرفة تأثير لاكتات الصوديوم كمحفز للنمو على كبد بنكرياس جمبري المياه العذبة ماكروبريكيم روزنبيرجي . غذي الجمبري على خمسة تركيزات للاكتات الصوديوم (١ ، ١,٥ ، ٢ ، ٣ و ٤ جرام كل كيلوجرام من الوجبة) لمدة ١٠٣ يوم .

أوضحت النتائج أن الخلايا المخزنة للدهن (R-cell) و الخلايا المفرزة (B-cell) كانت أكثر حساسية في كل التركيزات ، حيث أظهر تركيز ١% بعض التغيرات النسيجية القليلة ، بينما عند تركيز ١,٥% لوحظ أن الخلايا زاد حجمها و كثرت بها الفجوات . عند تركيز ٢ % زادت الفجوات أكثر و تراكمت فوق بعضها البعض . أما عند تركيز ٣% و ٤% فقد زاد عدد الخلايا المفرزة الغير طبيعية و ضمرت الخلايا الليفية (F-cell) . كذلك ظهرت الخلايا المخزنة للدهن بها فجوات أكثر و حدث تغلظ لأنيبيبات الغدة. نستخلص من هذه الدراسة أن التركيز النموذجي للاكتات الصويوم كمحفز للنمو هو ١% لأن هذا التركيز له تأثيرًا طفيفًا على الأنسجة كما أنه الأفضل للنمو، وذلك ما اكدتها دراسات بيولوجية أخرى مكملة لهذا البحث.