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Effect of Temperature on Hatching and Larval Development and Mucin Secretion in Common Carp, *Cyprinus carpio* (Linnaeus, 1758)

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Abstract: Eggs of common carp were naturally fertilized and incubated at different temperatures 20,24,27,35 and 38°C for a period of 70 h of fertilization The present results indicated that the optimum percent of healthy eggs was 77% at temperature of 27°C, followed by 59% at temperature of 30C°. However, no eggs were survived to hatch at neither 20°C nor 38°C. The hatching rate at 27°C and 30°C increased than the other treated groups. The lengths of newly hatching larvae were 3.3±0.015 mm at 24°C and yolk volume ranged from 0.421±0.005 to 0.676±0.005µl. It likely seems that, the yolk volume of newly hatching larvae varied inversely with increasing of water temperature. The growth of larvae increased at the optimum temperature from 27-30°C. Meanwhile, the effect of temperature ranges on the ontogeny of mucous cells showed that the optimum temperature from 24-30°C increased number and density of mucous cells in both of body surface and the alimentary canal. Early life stage larvae (20 h-old) exposed to 24°C showed neutral mucous substance in the buccal cavity then changed to acid mucous substance after three days post hatching. In skin of larvae (14-day old) exposed to 27°C, both neutral and acid mucous substances are located at the epidermal layer of skin. The goblet cells of larvae (21-days old) treated with 27°C are composed of acid mucous substance which increased in number and size towards the posterior intestine. It can be concluded the temperature has an important effect on hatching, growth, protective role of mucin secretion in common carp and may be applied to improve carp larvae rearing at hatchery system.

Key words: Temperature · Fertilization · Hatching · Survival rate · Histochemical examination · Cyprinus carpio

INTRODUCTION

Temperature is the main environmental factor governing the development of fish eggs [1]. It determines certain morphological feature, hatching rate and the behavior of larvae [2]. Fish farmers sometimes keeps nursery pond very shallow in order to help them heat up quickly. This may, in turn cause a considerable increase in the dial variation of water temperature. These variations are also clearly during characteristic weather changes during juvenile carp development in late spring [3,4].The temperature requirement varies among species and even for various developmental stages (e.g. spawning, embryonic larval and adult development) of given species [5,6]. In addition, temperature is known to influence the efficiency of yolk utilization [7, 8], it was reported that the growth rate increase with increasing of water temperature, but when the temperature becomes superoptimal, it has a negative effect [9-11]. Common carp, *Cyprinus carpio* is a fast growing fish with worldwide distribution and is reared in a polyculture of fresh water fishes in Egypt. There is a lack of adequate knowledge about the optimum rearing condition, especially for with temperature requirement of eggs and larvae [1].

The main objective of this study was to detect the influence of water temperature on eggs hatching as well as on larval growth and survival rate in common carp larvae, whereas few investigations were carried out in this respect Furthermore, the influence of temperature on the structure and activity of mucous cells in the alimentary canal and body surface of larval using histochemical techniques was another target of this study.

MATERIALS AND METHODS

Experimental Protocols: The experimental study was carried out at El-Serw Fish Researches Station during the spawning season of common carp, *Cyprinus carpio*, from

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21 April till 30 May, 2008. Seminatural spawning was carried out by using egg receptacles, since one female spawned with one male and sometimes two females with one male spawned together at cement ponds depending on the condition of fish. The body weight of parents used in spawning were recorded (1.5-2 kg for females and 1-1.25 kg for males).24 cement ponds were used,each has an area of 5×5 m^2 and the depth of water at each pond was not increased than 40 cm . The water temperature during the period of spawning ranged from 23-25°C. After the spawning occurred, small pieces of egg receptacles (10×10cm) containing fertilized eggs for experimental study were distributed into six glass aquaria

Eggs Incubation: Each glass aquarium contained approximately of 400 fertilized eggs. Water in aquaria were assigned to temperature treatments of 20, 25, 27, 35 and 38°C. At stocking, the temperature above the ambient medium was thermostatically controlled by electric heaters adjusted with thermostat, while temperature from 20°C and less than 23°C was monitored by adding fresh cold water. The water temperature was monitored every two hours. The light regime was to optimized at 12 h day/12 h night by using an electric lamp for lighting and by covering the pond with black screen for darkling. Each treatment had three replicates. The temperature fluctuation was within the range ±0.2°C. The percentage of healthy embryonic development during a period of 70 h of fertilization was calculated. Dead eggs were removed and were taken into a consideration. The interval from hatching after fertilization and the initial lengths of newly hatching larvae were recorded. The percentage of hatching and survival rates of larvae after 72-120 h of fertilization as well as the average of growth rate after 70 days post hatching was calculated. The characteristics of medium were adjusted to become (PH: 7-7.5), dissolved oxygen (5.5-6.5 mg/l) was also adjusted by replacing half volume of the culture with water aerated at same the temperature.

Feeding larvae: After three days post hatching, the larvae fed with culture contained live zooplankton collected from natural habitat. Three weeks later of post hatching, the artificial food containing on 25-30% protein was supplied twice a day. The experiment was terminated after 70 days post hatching.

Samples Collection: Samples were collected after 20 h as well as, 3, 7, 14, 21 and 30days of post hatching for histochemical evaluation. Larvae less than 6 mm in total length were directly fixed in bouin's fluid. However, a large specimen was decalcified with 5% nitric acid for about 12 h to remove any bone traces, then washed with distilled water and fixed in bouin's fluid as a usual manner.

Histological and Histochemical Procedures: After the larvae were fixed in bouin's fluid for about 48 h, they dehydrated in series of ethanol, cleared in xylene and finally embedded in paraplast wax (m.p. 56-58°C). Sagittal and serial transverse sections were cut at 5-6 microns thickness and finally stained with haematoxylin and counterstained with eosin [12]. For histochemical studies, the procedures were used to detect nature of carbohydrate materials. For mucous cells, the selected slides of the intestine and body surface from serial sections were stained with periodic acid Schiff (PAS), [13] and Alcian Blue (AB), [14.15].

Statistical Analysis: The statistical analysis was carried out [16] and Microsoft Excel, 2003.

RESULTS

Effect of Temperature on Fertilized Eggs: The optimum percent of healthy eggs (77) was obtained at temperature of 27°C followed by 30°C (59). Egg incubated at 24°C (control group) showed mortality rate of 50%, hence unhealthy eggs were died and changed into white color at temperature of 20°C and 38°C as shown in Table 1.

Table 1: Effect of temperature on embryonic development of fertilized common carp eggs during 70 h post fertilization.

Experiment No.	Temperature (°C)	Time (h) 8 h	16 h	24 h	32 h	40 h	48 h	56 h	64 h	70 h
1	20	0	0	0	0	0	0	0	0	0
2	24	85	80	77	72	66	63	59	52	50
3	27	98	95	92	88	87	85	81	80	77
4	30	90	88	85	81	77	72	69	61	59
5	35	61	55	48	37	35	31	25	17	15
6	38	15	5	0	0	0	0	0	0	0

• Number of eggs in each experiment was 400
• In experiment no.1, unhealthy eggs changed into white color and no embryonic development was observed
• In experiment no.6 the embryos were died after 16 h of eggs incubation

Table 2: Effect of temperature on the time (hr) of hatching eggs of Common Carp

Experiment no.	Temperature (°C)	Time to hatch (hr)
1	20*	>120
2	24	96-120
3	27	72-77
4	30	80-88
5	35**	90
6	38*	>120

* No hatching occurred at 20°C and 38°**very few hatching larva were counted at35°C

Table 3: Effect of temperature on percentage of hatchability and survival rates of common carp larvae after 72-96 h of fertilization

	Temperature (°C)					
Parameters	20	24	27	30	35	38
Hatching rate	0	48.0±0.118 (190)	74.33±0.232 (298)	54.20±0.273 (220)	2.5±0.129 (10)	0
Survival rate	0	38.0±0.281(75)	72.00±0.556 (215)	52.33±0.191 (115)	0	0

No hatching occurred at 20°C and 38°C more than 120 h of incubation.

No of fish is between the brackets.

Table 4: Effect of incubation temperature on the average lengths, of hatching carp larvae and yolk volume (µl) after 72-96 h of fertilization. (Mean +SE))

Experiment no.	Temperature (°C)	Average of lengths (mm)±SE	Yolk volume (µl)
1	24	3.3±0.015	0.676±0.005
			0.590±0.006
			0.421±0.005
2	27	3.7±0.021	0.556±0.008
			0.346±0.009
			0.323±0.006
3	30	3.6±0.032	0.6133±0.008
			0.373±0.005
			0.346±0.007
4	35	3.466±0.011	0.646±0.007
			0.556±0.006
			0.423±0.009

The hatching period (time required to hatch after fertilization) inversely with temperature, hence it was 96-120 h after incubation eggs with 24°C. This time ranged from 72-77 h after incubation at 27°C. No hatching occurred for eggs incubated at 20°C and 38°C during a period of 120 h of incubation as shown in Table 2.

Hatchability: No hatching occurred at temperatures of 20 and 38°C and the lowest value of hatching was recorded at 35°C. At optimum temperature (24-30°C), the hatching rate increased and ranging from 48.0±0.118, 74.33±0.232. The survival rate of newly hatching larvae increased at 27 and 30°C. However the lowest value of survival rate (38.0±0.281) was obtained at 24°C as recorded in Table 3.

Length of newly hatching larvae: The average lengths of newly hatching larvae was 3.3±0.015 mm at 24°C and the yolk volume ranged from 0.421±0.005,0.676±0.005 µl. The yolk volume of larvae varied inversely with temperature. The lengths of hatching larvae decreased as the temperature increased up to 35°C. The yolk volume was not reduced and ranged from 0.423±0.009, 0.646±0.007 µl at an average and the length of larvae were 3.46±0.011 mm at an average as in Table 4.

Growth and survival rate (70 days old): After exposed to the newly hatching larvae to various degrees of temperature for a period of 70 days of post hatching, the results showed that at 27°C, the growth was significantly different from the other groups either at 24 or 35°C($P<0.05$)as in Table 5.

Table 5: Effect of temperature on the average of growth±SE of common Carp Larvae, *Cyprinus carpio* after a period of 70 days of posthatching

	Temperature (°C)			
Parameters	24 control	27	30	35
Average of growth in length (cm)	2.571±0.061a	3.00±0.216b	2.942±0.120	2.428±0.162
Average of growth in weight (g)	0.3142±0.029 (25)[a]	0.660±0.057(25)[b]	0.614±0.053 (25)	0.3667±0.032 (25)

The number of measured fish larvae are between the brackets.

[a]Significance differences was detected (P<0.05) [b]No. significant different was detected (P>0.01)

Table 6: Effect of temperature on a percentage of survival rate of common carp larvae throughout 70 days of post hatching.

		Time (days)									
Experiment No.	Temperature (°C)	7	14	21	28	35	42	49	56	62	70
1	20	51	47	41	27	22	0	0	0	0	0
2	24	80	77	72	67	61	55	51	46	41	38 (95)
3	27	97	92	90	87	84	82	78	75	72	70 (175)
4	30	91	90	88	85	82	78	76	68	65	63(153)
5	35	61	57	53	46	41	35	30	0	0	0
6	38	32	26	8	0	0	0	0	0	0	0

The number of survival fish up to 70 days of post hatching is between brackets

At temperature of 20°C, the survival rate decreased gradually and reached to the lowest value (22%) after 35 day post hatching and then this value of morality rate reached to 100% after a period of 36 days. After raising temperature to 38°C, the survival rate was 8% after 21 days of post hatching and the mortality rate was recorded 100% after28 days under the same temperature. The optimum survival rates were recorded as 70 and 63% at temperatures of 27 and 30°C as shown in Table 6.

Histochemistry of Mucous Cells: At the early life stage (25 h old) post hatching, the buccal cavity exposed to before 24°C consists of a thin layer of stratified squamous epithelium. The mucous cells appeared round shaped, small in sizes and stained positively with the PAS reaction (Fig. 1a). After exposure of larvae to 27°C (3 days-old) the buccal cavity showed two types of mucous cells. The first is less abundant cells contained neutral mucous substance and the second is more numerous cells contained acid mucous substance (Fig. 1b). In the buccal cavity of fry exposed to 30°C (3days-old), the mucous cells appeared at different shapes and sizes containing acid mucous substance (Fig. 1c). These cells increased in both number and size with the progressive development of larvae (21 days-old) and contained basal flattened nuclei and had large vacuoles (Fig. 1d). The mucous cells in the skin (14 days-old larvae) following exposure to 27°C appeared as round shaped positively stained with PAS reaction and contained neutral mucosubstance and few of these cells contained acid mucosubstance after stained with alcian blue (pH 2.5). These cells appeared as large vacuoles after stained with haemtoxylin and eosin and appeared towards the outermargin of epidermal layer (Fig. 2 a and b). The alimentary canal appeared as straight tube at one day of post hatching larvae. The yolk sac occupies the anterior region on the ventral side of larva. At the early life stage of larvae (3days-old and 3.2 mm in length) the oesophageal wall can be distinguished from pharynx by a great number of mucus-secreting cells. After fourteen days of hatching, the oesophageal wall of larvae of 27°C group is lined by simple columnar epithelial cells containing ovoid-mucus secreting cells which open towards the lumen of the oesophagus and appeared as empty vacuoles after stained with haematoxylin and eosin (Fig. 2c). The intestinal wall of fry at early life stage (3days-old) is consisted of mucosa containing few number of mucous secreting cells (Fig. 2d) and (Fig. 3a). After exposure to 27°C, few of mucous cells in the intestine appeared as round-shaped contained acid mucous substance after stained with alcian blue and some of these cells reacted positively with PAS and contained neutral mucous substance (Fig. 3 b and c). The intestinal wall of larvae in 30°C group appeared as more or less round-shaped that located towards the outer border of mucosal folds. These cells contained acid mucous substance positively reacted with Alcian blue (pH 2.5)

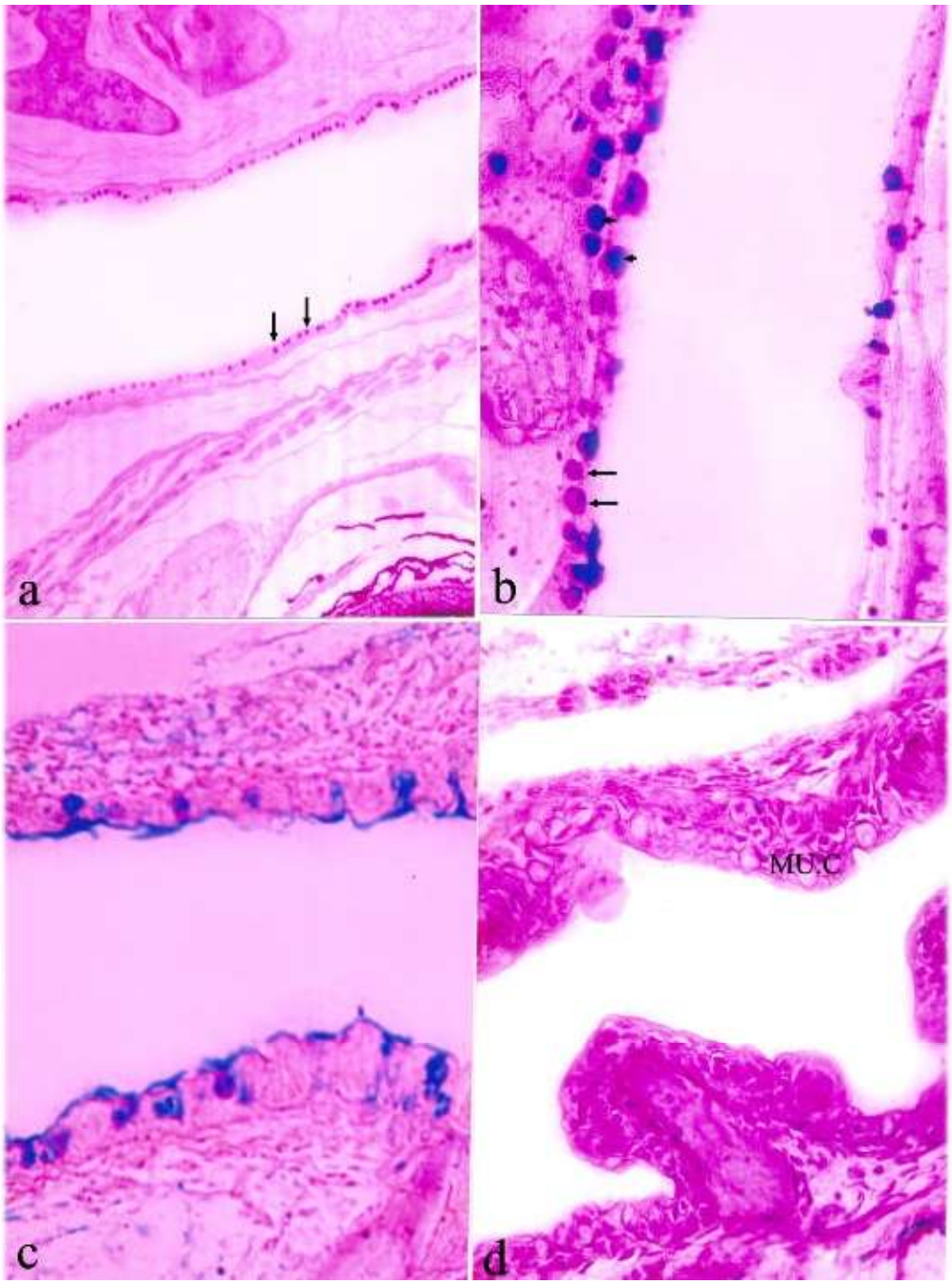

Fig. 1: a) Sagittal section in twenty hours of post-hatching larvae exposed to a temperature of 24°C, stained with AB-PAS, showing the mucous cells in the buccal cavity (Mu.c) X400. (b) and (c): Sagittal sections at the buccal cavity (3 days-old larvae) in 27°C and 30°C larvae, stained with AB-PAS, showing the mucous cells contained less neutral mucous substance (Arrowheads) and more numerous acid mucous substance (arrows), beside the taste bud(Tb). X400. (d) Sagittal section of buccal cavity (21day old larvae) exposed to 27°C, stained with haematoxylin and eosin, showing the mucous cells appeared as empty vacuoles (Mu.c), with increased in both number and size. X300

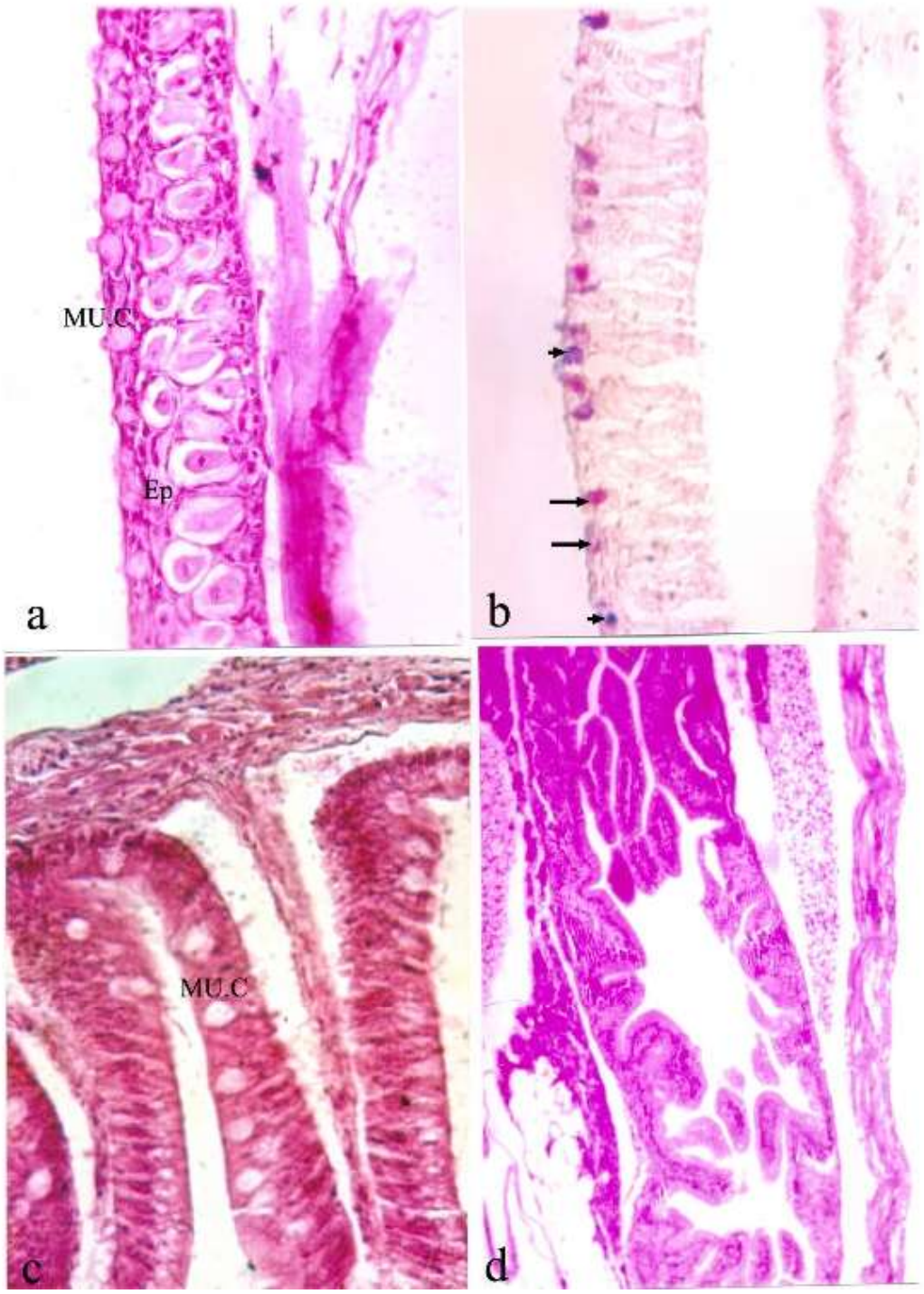

Fig. 2: a) Sagittal section of larvae skin (21-days old) exposed to 27°C,stained with haematoxylin and eosin, showing the skin is composed of epidermis (Ep.) and a thin layer of derms (D.), the round-shaped mucous cells are located at the outer margin of epidermal layer (Mu.c.) X400. b. The mucous cells in the skin, stained with AB-PAS either acid (Arrows) or neutral mucosubstance is observed (Arrowheads) X400. C: Sagittal section of esophageal wall of larvae (14 days-old) exposed to temperature 27°C, stained with haematoxylin and eosin, showing the mucosal folds contained numerous ovoid mucous cells (Mu.c.) X400.d) Sagittal section in the intestine of larvae (3 days-old) following exposed to 24°C, stained with hematoxylin and eosin, showing the mucosal folds contained few mucous cells. X 300

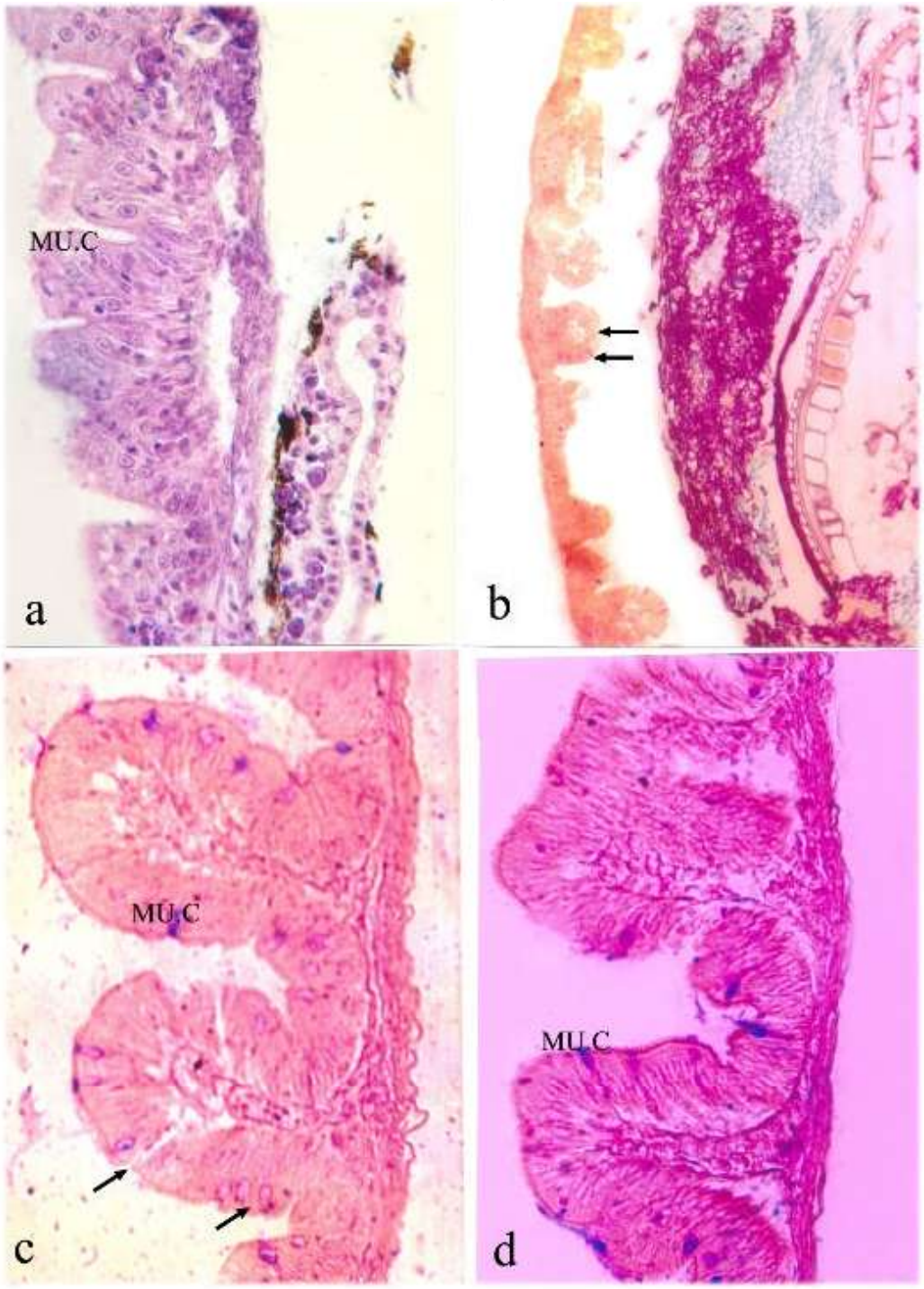

Fig 3: a) Magnified portion of Fig2d, stained with haematoxylin and eosin, showing few number of mucous cells (Mu.c.) at various levels of mucosal folds. X400. b). Sagittal section in the intestine of larvae (3days-old) exposed to 27°C, stained with AB-PAS-OG, showing , neutral mucous substance which are reddish in color and the remainder part of food in the lumen of intestine . X300. C: Sagittal section in the intestine of larvae (3days-old exposed to 27°C, stained with AB-PAS showing the mucous cells are composed of acid mucosubstance (Mu.c) and some of these cells appeared empty (Arrows). X400. d): Sagittal section in the intestine of larvae (3days-old) exposed to 30°C, showing increase number of mucous cells (Mu.c) at the outer border of mucosal folds. X400

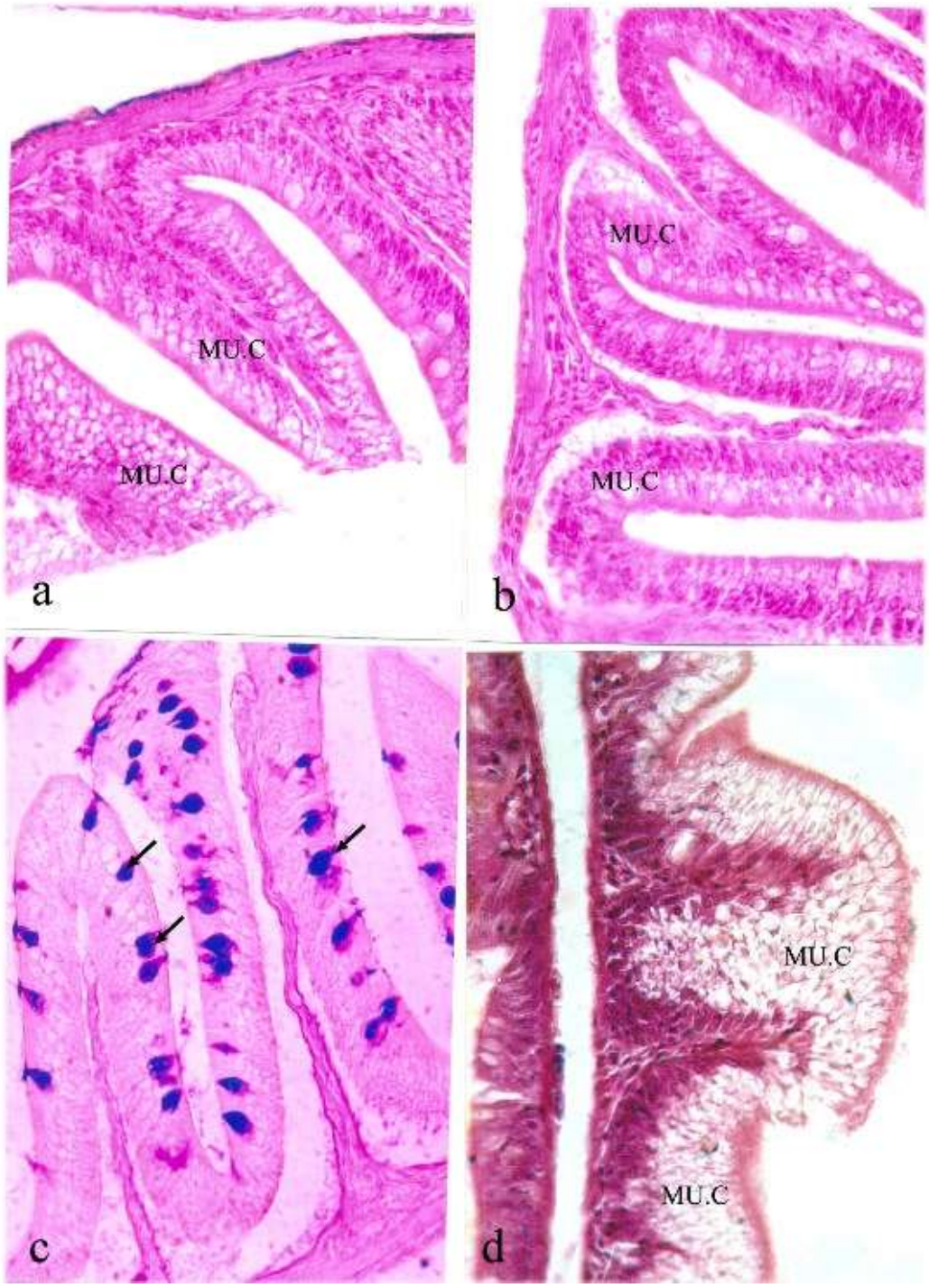

Fig 4 a): Sagittal section in the intestine of fish (21days-old) exposed to 24°C, stained with hematoxylin and eosin, showing simple columner epithelium cells intermingled with a moderate number of mucous cells (Mu.c.) .X400. b: As in section (a) an increase number of mucous cells (Mu.c.) were observed after the fish exposed 27°C. X400. c: section stained with AB-PAS, the mucosal folds contained acid mucosubstance of goblet cells (Arrows) towards the lumen of mucosal folds. X400. d: Sagittal section in the intestine of fish (21days-old), stained with hematoxylin and eosin exposed to 35°C, showing a large number of mucous cells (Mu.c.) covering a large area at the outermargin of mucosal folds X300

(Fig. 3d). In 21 days old samples, the mucosa of larvae after exposure to 24°C has long and branched into folds with simple columnar epithelium intermingled with mucous cells (Fig. 4a). These cells in 27°C group increased in both size and number and appeared as goblet cells (Fig. 4b). The mucous cells contained neutral mucosubtance and were few in number, however more numerous cells contained acid mucosubstance after staining with Alcian blue were obvious (Fig. 4c). After exposure of fish (21 days-old) to 35°C, the mucosal folds appeared in abnormal structure and the mucous cells increased in both size and number, covering a large area at outer margin of mucosal folds (Fig. 4d).

DISCUSSION

The eggs and yolk sac of fry common carp, *cyprinus carpio* were incubated in an experimental hatchery system at different degrees of temperature. The embryonic development of eggs, hatchability and growth of yolk sac of fry were studied under controlled conditions. It has shown that the time from fertilization to hatching decreased with increased temperature. These finding agree with the results obtained from other fish species [17-19]. In the present work, the maximum percent of larval hatching increased as temperature increase (24-30°C). These results indicated that the optimum temperature for hatchability of this species lies between 24 and 30°C. These finding corresponds to the environmental of temperature during the period of natural reproduction. These finding agree with the results from other species [1]. It likely seems that the temperature affects the tolerance level of eggs, since the optimum number of healthy eggs was 77% at temperature of 27°C followed by 59% at temperature of 30°C. Similar results were observed on other species [20]. In the present study, neither eggs survived to hatch at 20°C nor 38°C. Failure of eggs of carp to hatch at temperatures 20 and 38°C in three successive experiments suggests that this temperature is outside the tolerance limit for development of carp eggs, however, few of eggs were survival and hatched at 35°C. The temperature is known to influence embryonic development and affects survival and efficiency of yolk utilization [7, 8]. In the present study, the lengths of newly hatching larvae exposed to 24°C was 3.3±0.015 mm and the yolk volume ranged from 0.421±0.005 and 0.676±0.005 µl. It likely seems that, the yolk volume of larvae varied inversely with temperature. Many studies have shown that the incubation with temperature influence the size of newly hatching larvae, since at higher temperature, the length of newly larvae are generally being shorter [21, 22]. However, [23] found that eggs incubated at 13°C produced significantly longer larvae than eggs incubated at the lower temperature. The yolk volume in newly hatched carp larvae under the present study was significantly larger at cold temperature. Furthermore, the high mortality prior for hatching during the early embryonic development of carp eggs which occurs at early hours after fertilization at all experiments coincide with [24], who attributed the cause of mortality for an oxygen deficiency during a period of high oxygen demand or inhibition of the production of enzymes involved in hatching [25]. In addition, temperature may be the single most important factor determining the growth rates of early life stages of fish [21, 26]. The mortality rates during the early life stages of larvae are generally high [27]. In present study, the cause of mortality observed during the first days after the yolk-sac absorbed frys may partly be explained by either by stress during handling. Furthermore the mortality seems to be associated with more critical period in which the carp larvae failed to initiate and maintain successful feeding in switching from endogenous to exogenous nutrition. In addition, the growth of carp larvae that exposed to either 27 or 30°C was obviously different than from the other treated groups either with 24 or 35°C. After the temperature elevated to 38°C, the growth decreased and survival rate was 8% after 21 days of post hatching, thereafter, the mortality rate was 100% after 28 days of post hatching at some circumstance of water temperature. The investigated growth rate increased with increasing of water temperature, but when the temperature becomes super optimal, it has a negative influence [11]. In addition, the temperature influence metabolic processing and is the single most important factor that determines growth rates in fish [28]. It is important to state that influence of temperature on growth needs further studies in correlation with metabolic processing activity of carp larvae. The mucous coat of fish forms the primary barrier against infection [29] and fish skin mucus may inhibit the growth of bacteria [30]. In addition, the mucus plays an important role at various food processing activities [31].In the present study, the effect of temperature on the alimentary canal and body surface of developed larvae, showed that the optimum temperature ranges from 24-30°C as indicated by increased number and density of mucous cells. Similar results were observed on other species [32]. The authors added that exposure of *O.niloticus* larvae to exogenous hormonal treatment and temperature affects the activity of mucous cells. In the present results, an increase in density

and number of mucous cells in the body surface and alimentary canal of carp larvae as well as changes of mucous cells composition attributed to raising of water temperature as also indicated by [15,32].

It could be concluded that the optimum temperature ranges from 24-30°C plays an important role for improvement hatchability, growth, food intake and defense mechanism during the period of the carp larval development. These findings may be applied to protect and improve carp larvae in rearing at hatchery system.

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