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Effect of exogenous hormonal treatment on growth, survival and mucous cell activity during larval development of *Oreochromis niloticus*

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Abstract

In *Oreochromis niloticus* larvae treated with cortisol (C0.1 ppm); L- Thyroxin ($T_{0.05}$ or T0.1 ppm) and 75 mg/kg of food 17α Methyl testosterone ($T_{0.05}$ ppm + MT, or $T_{0.1}$ ppm + MT) to accelerate larval growth. Treated larvae gave also a significantly higher survival rate than that of control. The treatment with exogenous hormones improved larval growth, since increasing thyroxin from (T0.05 ppm or T0.05 ppm + MT) to (T0.1 ppm or T0.1 ppm + MT), the larvae displayed prominent increase in both their length and weight. These observations suggest that therapy (thyroxin, cortisol and MT) may have a practical utility in fish culture. Exposure of *O. niloticus* larvae to exogenous hormonal treatment enhanced the production of mucous cells in digestive tract. There was an increase in the number of mucous cells in the elementary tract as well as quantitative changes of the mucous composition, the enhancement of the digestive function, improved food utilization and activated the protective role of mucins. Both the synthetic and secretory activities were increased in the mucosal layer of the numerous folds in the stomach of the treated larvae with thyroxin for seven dph whereas, treated with 17α MT for 21 day post hatching (dph), as reflected by staining affinity with periodic acid Schiff reagent and Alcian blue techniques. In treated *O. niloticus* larvae with high dose there was an increase in the number of mucous cells in various parts of digestive tract, which contains acidic mucosubstances when compared with control group.

1. Introduction

Many regulatory compounds such as hormones, neurotransmitters, and mRNAs which encode for growth factors and other compounds are maternally derived and deposited into the yolk of vertebrate eggs (Brown & Nunez 1994). Thyroid hormones are important regulatory hormones that increase epidermal mitotic rate by controlling the synthesis of specialized proteins during cell differentiation within the digestive system, in the formation and inflation of the swim bladder, and in the development of muscle tissue (Hourdry, 1993). They also frequently reported to have a clear effect on improving larval health and survival rate. Thyroid hormone treatments increase larval survival rate in walleye and gold stripped amberjack (Hey and Farrar, 1996 and Tachihara *et al*., 1997). Thyroid hormones are deposited into eggs against the concentration gradient during vitellogenesis (Bjornsson *et al*., 1998). Another important regulatory hormone in teleosts is cortisol, which is involved in the maintenance of hydromineral balance, osmoregulation and glucose metabolism (Mc Cormick, 1995). According to Bhandari *et al*. (2006) and Marigani *et al*. (2009) sex reversal affect growth performance of tilapia (*Oreochromis mossambicus*). The dose rate of 75 mg/kg^{-1} 17α Methyl testosterone gave the maximum gain in body weight. Peduel & Ron (2003) indicated that acclimatization of juvenile of the white grouper *Epinephelus aeneus* by treatment with cortisol produced fish that can flourish in brackish water, with no determinable chemical factors. Koven (2003) indicated that cortisol and thyroid hormones play a major role in regulating many developmental processes that occur during metamorphosis. Fouz *et al.* (1990) indicated that the epidermal mucous layer constitutes the primary biological interface between fish and the aqueous environment. The mucous coat of the fish presents the primary barrier against infection. Tibbetts (1997) and Gallagher *et al.* (2001) pointed out that the mucous cells play an important role in the various food processes. The present work is aimed to study the effect of exogenous treatment with thyroxin, cortisol and 75 mg/kg 17α MT on growth, survival and mucous cell activity during larval development of the *Oreochromis niloticus* fish.

2. Material and methods

The present experiment was carried out at marine hatchery, National Institute of Oceanography and fisheries throughout the period from April to October 2007.

2.1. Natural spawning and production of larvae

Oreochromis niloticus spawners were obtained from Edku east of Alexandria. Fish brood were kept in fiberglass tanks (3000L volume) for 10 days for acclimatization (150 -250 g in total weight and 15 to 21 cm for total length) the sex ratio of spawners was three females to one male.

2.2. Experimental design and treatment

2.2.1. Chemicals

Thyroxin (T_4) was used Galaxo chemical products; under the commercial name Eltroxin tablets each containing 0.05 mg anhydrous thyroxin sodium. Cortisol was used as " Soluo –Cortef " which is a commercial preparation (EIPICO) of hydrocortisone sodium succinate in the form of ampoule (100 mg /2ml) of the hormone and 17α Methyl testosterone.

2.2.2. Treatment

Fifteen glass aquaria (100 X 50 X 40 cm) were used for the experiment each containing 50 liter of fresh water. Newly hatched larvae were divided into three groups (three aquaria for each group). The density of larvae was about 500 larvae /aquarium i.e. 10 larvae /L.

- The first group was used as control.
- The second group ($T_{0.05}$) was treated with thyroxin 0.05 ppm and cortisol (C 0.1) ppm for seven days.
- The third group ($T_{0.05}$ + C 0.1 + MT) was treated with thyroxin 0.05 ppm and cortisol 0.1 ppm for seven days and 75 mg/kg for 21 days 17α Methyl testosterone (Phelps and Cerezo, 1992).
- The fourth group ($T_{0.1}$ + C 0.1) was treated with thyroxin 0.1 ppm and cortisol 0.1 ppm for seven days.
- The fifth group ($T_{0.1}$ + C 0.1+ MT) was treated with thyroxin 0.1 ppm and cortisol 0.1 ppm for 7 days and 75 mg /kg 17α Methyl testosterone for 21 days.

Larvae were fed on Artemia sp newly hatched nauplii, then dry food with 35% crude protein was used for feeding in a daily fixed rate at 5% of the total fish weight.

2.2.3. Larval sampling and processing

Ten *O. niloticus* larvae were randomly sampled at the first day of hatching (newly hatched), 2 ,4 ,6 ,8 ,14 ,21 ,28 ,35 ,42 ,49 ,56 and 63 day post hatching (dph) then, anesthetized using phenoxyethanol (1 ppm) and placed on paper towels for about 3 seconds to remove most of the adhering water, measured to nearest mm., weighed to nearest gm and fixed in 10% formal saline 's fluid for histochemical study. After fixation and dehydration through series of ethanol, they were cleared in Xylene and embedded in paraffin wax (M. P. 58 -65 °C). Serial transverse and sagittal sections ,5 µm thick , were cut and stained with Alcian blue (AB) / Periodic acid Schiff reagent (PAS) at pH 1.0 according to Culling (1978). Positive reactions for mucous cell histochemistry at AB/PAS at pH 1.0 were blue for acid mucin red for neutral mucin and reddish purple for a combination of neutral and acid mucins.

Statistical analyses were done by Microsoft windows (2000) Excel program. Factorial design by ANOVA test was applied (Gatline *et al*., 1986)

3. Results and discussion

3.1. Effect of cortisol, thyroxin and methyl testosterone treatment on the growth and survival rate

3.1.1. Growth in length

The treatment program with thyroxin ($T_{0.1}$ ppm or $T_{0.05}$ ppm) and 75 mg /kg 17α Methyl testosterone ($T_{0.1}$ +MT or $T_{0.05}$ ppm +MT) and cortisol (0.1 ppm) is represented in Table 1.

Highly significant increase was recorded in the total length of the tilapia *Oreochromis niloticus* larvae treated with high dose of thyroxin (0.1ppm) at age 4,8,14,21,28,35,42,49,56 and 63 days post hatching ($p<0.1$), where significant increase for the 2 and 6 dph ($p<0.05$) .Similarly, Highly significant increase was noticed in the total length of *O. niloticus* larvae treated with low dose of thyroxin (0.05ppm) at age 14,21,28,35,42,49 and 56 dph ($p<0.1$), also noted significant increase in total length for larvae at age 2,4,6,8 and 63 dph. These results show that exposure to exogenous hormones have accelerated larval growth, since a higher increase in both, length and weight of larvae occurred during the experimental period. Similar reports showed that thyroid hormones accelerate growth and development in teleost larvae (Brown and Kim, 1995; Ansal and Kaur,1998; Power *et al*.,2001; Gavlik *et al*.,2002; Mousa, 2004; Assem, 2005 and Khalil *et al.*, 2006). Larvae treated with cortisol (0.1ppm) and high dose of thyroxin and 17α Methyl testosterone ($T_{0.1}$ +MT) exhibited a highly significant increase in total length of *O. niloticus* larvae at age 4,6,8,14,21,28,35,42,49,56 and 63 dph ($p<0.1$) while significant increase was noted at age 2 dph ($p<0.05$).

The results also revealed that, highly significant increase in total length of larvae *O. niloticus* treated with low dose of thyroxin and 17α Methyl testosterone (T0.05 ppm +MT) at age 6, 8,14,21,28,35,42,49 and 56 dph ($p<0.1$), whereas significant increase was noted in total length at age 2,4 and 63 dph ($p<0.05$).

Table 1. The effect of treatment with cortisol (C0.1 ppm), thyroxine (T0.1-T0.05 ppm) and 75 mg/Kgm 17αMethyltestosterone (T0.1+MT or T0.05+MT) on total length (mm) (mean ± SD) of *Oreochromis niloticus* larvae throughout the period from April to October 2006.

Age	Mean total length (mm)				
	Control	High dose T	Low dose T	High dose T+TM 75mg/Kg	Low dose T+TM 75mg/Kg
Days post hatching		$T_{0.1 + C\ 0.1\ ppm}$	$T_{0.05\ + C\ 0.1\ ppm}$	$T_{0.1ppm + C\ 0.1\ ppm}$	$T_{0.05\ + C\ 0.1\ ppm}$
0	8.1±0.01	8.1±0.01	8.1±0.02	8.1±0.02	8.1±0.01
2	8.4±0.01	9.3±0.03[b]	8.7±0.04[b]	9.7±0.01[b]	8.9±0.02[b]
4	9.0±0.02	9.9±0.05[a]	9.3±0.03[b]	10.9±0.05[a]	10.1±0.08[b]
6	9.8±0.03	10.2±0.08[b]	9.9±0.07[b]	12.3±0.09[a]	11.2±0.08[a]
8	13.0±0.09	15.3±1.03[a]	14.2±1.01[b]	16.7±1.05[a]	15.6±1.01[a]
14	13.6±0.08	16.1±1.03[a]	15.3±1.05[a]	17.8±1.01[a]	16.5±1.03[a]
21	14.3±1.01	18.3±1.1[a]	16.6±1.2[a]	20.5±1.1[a]	17.4±1.1[a]
28	16.2±0.9	24.2±1.3[a]	18.3±1.5[a]	26.3±1.3[a]	20.4±1.5[a]
35	19.1±0.8	28.5±1.4[a]	22.4±1.5[a]	30.3±1.7[a]	24.6±1.3[a]
42	23.3±0.7	33.4±1.8[a]	28.5±1.3[a]	34.5±1.9[a]	30.1±1.1[a]
49	28.2±0.8	35.4±1.7[a]	30.3±1.1[a]	36.5±1.07[a]	32.5±1.08[a]
56	32.4±1.01	38.3±1.3[a]	34.2±1.1[a]	40.01±1.1[a]	35.6±1.2[a]
63	36.1±1.01	41.3±1.1[a]	39.2±1.3[b]	43.5±1.5[a]	38.5±1.2[b]

Table 2. The effect of treatment with cortisol (C 0.1 ppm), thyroxine (T0.1-T0.05 ppm) and 75mg/Kgm 17αMethyltestosterone (T0.1+MT or T0.05+MT) on total wieght (mean ± SD) of *Oreochromis niloticus* larvae for seven days post-hatching, throughout the period from April to October 2006.

Age	Mean total wieght (gm)				
	Control	High dose T	Low dose T	High dose T+TM 75mg/Kg	Low dose T+TM 75mg/Kg
Days post hatching		$T_{0.1 + C\ 0.1\ ppm}$	$T_{0.05\ + C\ 0.1\ ppm}$	$T_{0.1ppm + C\ 0.1\ ppm}$	$T_{0.05\ + C\ 0.1\ ppm}$
0	0.033±0.01	0.033±0.03	0.033±0.01	0.033±0.01	0.033±0.03
2	0.066±0.01	0.592±0.01[a]	0.316±0.08[b]	0.601±0.02[a]	0.401±0.03[b]
4	0.325±0.03	1.032±0.3[a]	0.617±0.05[b]	1.121±0.3[a]	0.81±0.1[b]
6	0.569±0.05	2.141±0.8[a]	1.331±0.06[a]	3.291±0.3[a]	1.921±0.5[a]
8	0.736±0.1	3.212±0.9[a]	1.922±0.1[b]	5.321±0.4[a]	2.211±0.8[b]
14	1.132±0.3	5.122±1.2[a]	2.33±0.8[b]	7.231±1.01[a]	2.88±0.9[b]
21	1.821±0.2	6.331±1.1[a]	2.98±0.11[b]	8.141±2.01[a]	3.971±0.4[a]
28	2.033±0.5	8.913±1.3[a]	4.01±0.9[a]	10.717±1.1[a]	5.611±0.3[a]
35	3.158±0.7	10.213±0.82[a]	5.23±0.8[b]	12.121±2.01[a]	7.112±1.01[a]
42	3.737±0.2	11.721±2.01[a]	5.99±0.9[b]	13.313±3.1[a]	8.012±1.08[a]
49	4.325±0.5	13.513±1.1[a]	6.83±1.1[a]	15.121±2.1[a]	9.11±2.1[a]
56	4.983±0.4	15.315±1.9[a]	7.815±1.01[a]	16.981±1.2[a]	10.431±2.3[a]
63	5.731±0.7	17.621±2.1[a]	9.213±1.3[a]	19.41±1.1[a]	12.132±1.4[a]

3.1.2. Growth in weight

Larvae treated with cortisol (C 0.1 ppm) and high dose of thyroxin (T0.1 ppm) exhibited a highly significant increase of mean body weight than of control at ($p<0.1$). Moreover, treatment with low dose of thyroxin (T0.05 + C0.01 ppm) gave highly significant increase in average body weight greater than those of control ($p<0.1$) at age 6,28,49,56 and 63 dph ,whereas significant increase in average body weight was recorded at age 2,4,8,14,21,35 and 42 dph ($p<0.05$) as indicated in Table 2.

Highly significant increase was noticed in mean total weight (gm) of larvae treated with cortisol (0.1ppm) and high dose of thyroxin and MT ($T_{0.1}$ +MT) compared to control group ($p<0.1$). Moreover, treatment with low dose of thyroxin and MT ($T_{0.05}$+MT) gave highly significant increase in average body weight (gm) than those of control ($p<0.1$) at age 6,21,28,35,42,49,56 and 63 dph. Also the result revealed a significant increase in mean body weight (gm) was noticed at age 2, 4 and 8 dph ($p<0.05$). The response of *O. niloticus* larvae to thyroid hormone treatment was found to be dose-dependent. Similar findings were observed in milkfish *Chanos chanos* (Lam *et al*.,1985); Indian major carp *Cirrhina merigala* (Ansal and Kaur,1998); the grouper *Epinephelus coioides* (De Jesus *et al*., 1998); common carp *Cyprinus carpio* (Mousa *et al*., 2002) and Nile tilapia, *Oreochromis niloticus* (Salama, 2004). The present study indicated that no abnormalities were observed in *O. niloticus* larvae treated with thyroxin (0.05 ppm or

0.1 ppm) for 7 dph. These doses of thyroxin were appropriate for accelerating growth and improving survival rate. Similar results were obtained by De-Jesus *et al*. (1998), for the grouper *Epinephelus coioides,* using thyroxin T_4 at several concentrations (0.01, 0.1 and 1ppm). The results were agreement with Mousa *et al*. (2002) for *Cyprinus carpio,* and Salama (2004) for *Oreochromis niloticus* who used thyroxin (0.05 or 0.1 ppm) and cortisol (0.1ppm). In contrast, Huang *et al*. (1996) reported that treatment of stripped bass *Morone saxatilis* larvae with 100 and 50 µg/ml resulted in retarded growth and lower survival, compared with fish treated with 25 µg/ml of the hormone or with control. Nugegoda *et al*. (1994) indicated that the growth and survival of the larvae of the sea bass, *Lates calcarifer,* were depressed after treatment with relatively low dose of thyroid hormones. In addition, Nacario (1983) found that while T_4 with dose of 0.1 ppm accelerated yolk absorption in tilapia (*Sarotherodon niloticus), while* ,high dose (1ppm) of hormone did not improve growth and survival but instead, caused abnormal shapes in the pectoral fins as well as lordosis and scoliosis.

Table 3. The effect of treatment with cortisol (C 0.1 ppm), thyroxine (T0.1-T0.05 ppm) and 75 mg/Kgm 17αMethyltestosterone (T0.1+MT or T0.05+MT) on survival rate (%) (mean ± SD)of *Oreochromis niloticus* larvae throughout the period from April to October 2006.

Age	Percent of survival (%)				
	Control	High dose T	Low dose T	High dose T+TM 75mg/Kg	Low dose T+TM 75mg/Kg
Days post hatching		$T_{0.1 + C\ 0.1\ ppm}$	$T_{0.05\ + C\ 0.1\ ppm}$	$T_{0.1ppm + C\ 0.1\ ppm}$	$T_{0.05\ + C\ 0.1\ ppm}$
0	100	100	100	100	100
2	98 ± 1	98 ± 0.5	98 ± 0.5	98 ± 0.4	98 ± 0.7
4	94 ± 0.5	95 ± 0.7	95 ± 0.3	94 ± 1	94 ± 1
6	93 ± 0.8	94 ± 0.5	93 ± 1	91 ± 1	94 ± 0.5
8	90 ± 0.3	94 ± 0.9[a]	91 ± 1[a]	89 ± 0.5	92 ± 1[a]
14	87 ± 0.4	92 ± 0.5[a]	91 ± 0.8[a]	88 ± 0.8[a]	90 ± 0.8[a]
21	84 ± 1.1	92 ± 0.8[a]	89 ± 0.9[a]	87 ± 0.7	87 ± 1
28	78 ± 0.5	91 ± 1[a]	87 ± 1[a]	85 ± 0.3[a]	83 ± 0.7[a]
35	75 ± 0.3	90 ± 0.8[a]	85 ± 1[a]	83 ± 0.6[a]	81 ± 1[a]
42	73 ± 1	89 ± 1[a]	83 ± 1[a]	79 ± 0.5[a]	80 ± 0.9[a]
49	70 ± 0.7	89 ± 0.9[a]	82 ± 2[a]	77 ± 0.9[a]	79 ± 1[a]
56	68 ± 0.9	88 ± 1.1[a]	81 ± 1[a]	75 ± 1.1[a]	77 ± 0.9[a]
63	66 ± 1.8	87 ± 1.5[a]	79 ± 1.2[a]	73 ± 1.2[a]	75 ± 0.8[a]

C: cortisol
T: thyroxine
MT: 17αMethyltestosterone
[a] Highly significant when compared to control (p › 0.01)

3.1.3. Survival rate

Treatment with cortisol (0.1ppm) and high dose of thyroxin (0.1ppm) or high dose of thyroxin and 75 mg/kg 17α Methyl testosterone (0.1ppm +MT) and low dose of thyroxin (0.05ppm) or (0.05ppm +MT) improved with high significant survival comparing to control. Also, the results revealed that, the low mortalities rate occurred at high dose value of thyroxin (T0. 1) and MT. The treated larvae exhibited highly significant (P<0.1) survival rate for both high (T0.1) and low (T0.05) thyroxin treatment concentration at age 8,14,21,28,35,42,49,56 and 63 dph compared to control. Highly significant (P<0.1) survival rate was noted for high (T0.1) and low (T0.05) thyroxin and Methyl testosterone treatment concentration with the presence of cortisol (C0.1) at age 8,14,28,35,42,49,56 and 63 dph (Table 3). At the end of experiment the mean survival values for the control, high dose of thyroxin (0.1ppm) or (0.1ppm +MT), low dose of thyroxin (0.05ppm) or (0.05ppm +MT) in the peresence of cortisol (C0.1)were 66 ±1.8, 87 ±1.5, 73 ±1.2, 79 ±1.2 and 75 ±0.8 respectively at 63 dph.

The present results indicated that there was a positive effect of thyroxin treatment and17α MT in the presence of cortisol with dose 0.1 ppm on larval growth and survival rate of tilapia larvae. Moreover, significant increase was recorded in the survival rate of treated larvae compared to the control. Similar observations were obtained by Ayson & Lam (1993) in *Siganus guttatus,* Tanaka *et al*. (1995) in marine fish; Mousa *et al*. (2002) in *Cyprinus carpio* and Salama (2004) in *Oreochromis niloticus*, Assem (2005) in Red tilapia and Khalil *et al*.(2006) in *O. niloticus* .

3.2. Effect of cortisol, thyroxin and 17α methyl testosterone treatment on mucous cell activity

During development, there was a marked increase in the activity of mucous cells, detected by their strong affinity to Alcian blue and Periodic Acid Schiff reagent (PAS); on the second day after hatching there were weakly stained mucous cells in the digestive tract of

normal larvae. Meanwhile, mucous cells in the buccal cavity appeared few in number and small in size (Fig.1A & B). In the digestive tract of larvae treated with cortisol 0.1ppm and high dose of thyroxin (0.1ppm) or (0.1ppm +MT) (Fig.1C&D) compared to cortisol with dose 0.1 ppm and low dose treatment (T0.05 ppm) or (T0.05 ppm +MT) (Fig.1E&F), the mucous cells increased in number and in the staining affinity of larvae treated with high dose comparing that with the low dose.

By progress in larval development, mucous cells of untreated larvae at age 4 dph in the buccal cavity were smaller in size degranulated and weakly stained with AB (Figure 2A). Mucous cell in the buccal cavity of larvae treated with thyroxin (T0.1 ppm) or (T0.1 ppm +MT), at age 4 dph, were larger in size and strongly stained with AB and PAS (Figure 2B&C). The activity of the mucous cells in the digestive tract of larvae treated with thyroxin 0.05 ppm and or (T0.05 ppm +MT) (Figure 2 D&E) was lower than that of the high dose. In present results, there was an increase in the number of mucous cells in the alimentary tract as well as qualitative changes of the mucous composition during treatment with exogenous hormones. Similarly, the onset of initial gut formation and initial somatic pigmentation was accelerated in newly hatched larvae of Pacific thread like fish (*Polydectylus sexfilis)* exposed to triiodothyroxin (T_3; 2.6 ppm) and cortisol (0.1 ppm) by immersion for one hour (Brown and Kim, 1995). Also, thyroxin treatment accelerates stomach organogenesis in early metamorphosis of summer flounder (*Paralychthys dentatus).* Inhibition of T_4 synthesis with thiourea (T_u) delays stomach development (Soffientino & Specker, 2000). On the other hand, De-Jesus *et al*. (1990) indicated that cortisol (0.1 ppm) enhanced the stimulating action of T_4 (0.01 ppm) on Japanese flounder (*Paralichthys olivaceus)* larval metamorphosis. Flik & Perry (1989) pointed out that in rainbow trout (*Salmo gardneri)* cortisol exerts hypercaemic effects by stimulating Ca^{2+}. The role of thyroxin was reported by Higgs *et al*. (1992) and Ansal &Kaur (1998) to stimulate appetite and food utilization directly or indirectly by stimulating growth hormone secretion which in turn, results in higher food conversion efficiency. Fifteen dph, some of mucous cells appeared near the surface of the buccal cavity and contained mucosubstances with strongly stained and larger cells at all groups treated with high dose of thyroxin (T0.1 ppm) or (T0.1 ppm +MT) in compared to control group (Figure 3A,B&D). Moderate amount of mucous cells were recorded in group treated with (T0.05 ppm) or (T0.05 ppm +MT) (Fig.3C&E) at fifteen days post hatching.

The goblet cells in the intestine of larvae treated with high dose of thyroxin (T0.1 ppm) or (T0.1 ppm +MT) at age 22 dph increased in number and contained large amount of acid mucosubstances when compared to control group (Figure 4A, B&D). Moderate number of goblet cells were noticed in the group treated with (T0.05 ppm) or (T0.05 ppm +MT) at age 22 dph (Figure 4C&E).

In present study, enhancement of the digestive function, induced by thyroxin and cortisol, led to the improvement in food utilization and activated the protective role of mucins. An increased rate of growth and reduced rate of mortalities were thus encountered, as formerly pointed out by Mousa *et al*. (2002) in case of *Cyprinus carpio.* Treatment of marine larvae with thyroid hormones would therefore be beneficial, since T_4 is known to stimulate the uptake of both proteins and fats, in the digestive tract as reported by Tanaka *et al*. (1995). Woo *et al*. (1991) and Higgs *et al.* (1992) recorded an improved appetite and food conversion efficiency as a result of higher intestinal enzyme activity in underyearlings of red sea bream *(Chrysophyrys major)* and salmonids after oral administration of T_3 respectively. While, Kumar *et al*. (1991) also observed better food conversion ratio following T_4 administration in *C. carpio*. Mucous cells are ones of the main factors in the stress response (Ottaviani *et al*., 1997; Mola *et al*., 2004 and Pepels & Balm, 2004), the histochemical detection of mucous secretion in digestive tract in which the gut associated lymphoid tissue (GALT) will differentiate, may suggest a role for mucous and goblet cells in early defense mechanisms in *O. niloticus,* before of establishment of cell-mediated immune responses in GALT. Recent findings suggest that a more primitive role for this peptide in vertebrates is as a dual hypophysiotropic factor both thyroid and the interrenal (adrenal) axes (Denver, 1999 and Boosre & Denver, 2004).

At age 32 dph, the mucous cells in the intestine of larvae treated with high dose of thyroxin (T0.1 ppm) or (T0.1 ppm +MT) in the presence of cortisol (C0.1 ppm)there are increase in size and number of goblet cells, which strongly stained with AB and PAS when compared to control group (Figure 5A,B&D). In addition, during this stage, some of the digested food was seen in the lumen of the intestine. The mucous cells in the intestine of larvae treated with low dose of thyroxin (T0.05 ppm) or (T0.05 ppm +MT), at age 32 dph, had a goblet cells while most of them were positively stained with both AB and PAS (Figure 5C&E). These cells contained moderate amount of acid mucosubstances.

The present results revealed that the treatment of *Oreochromis niloticus* larvae with exogenous hormones (thyroxin, cortisol and 17α Methyl testosterone) affects the activity of mucous cells. An increase in the number of mucous cells in the alimentary tract, as well as qualitative changes of the mucous composition occurred during the treatment with exogenous hormones. Similar observations were obtained in teleost larvae (*Cyprinus carpio* by Mousa *et al.* (2002), Red tilapia by Assem (2005) and *Oreochromis niloticus* by Khalil *et al*. (2006).

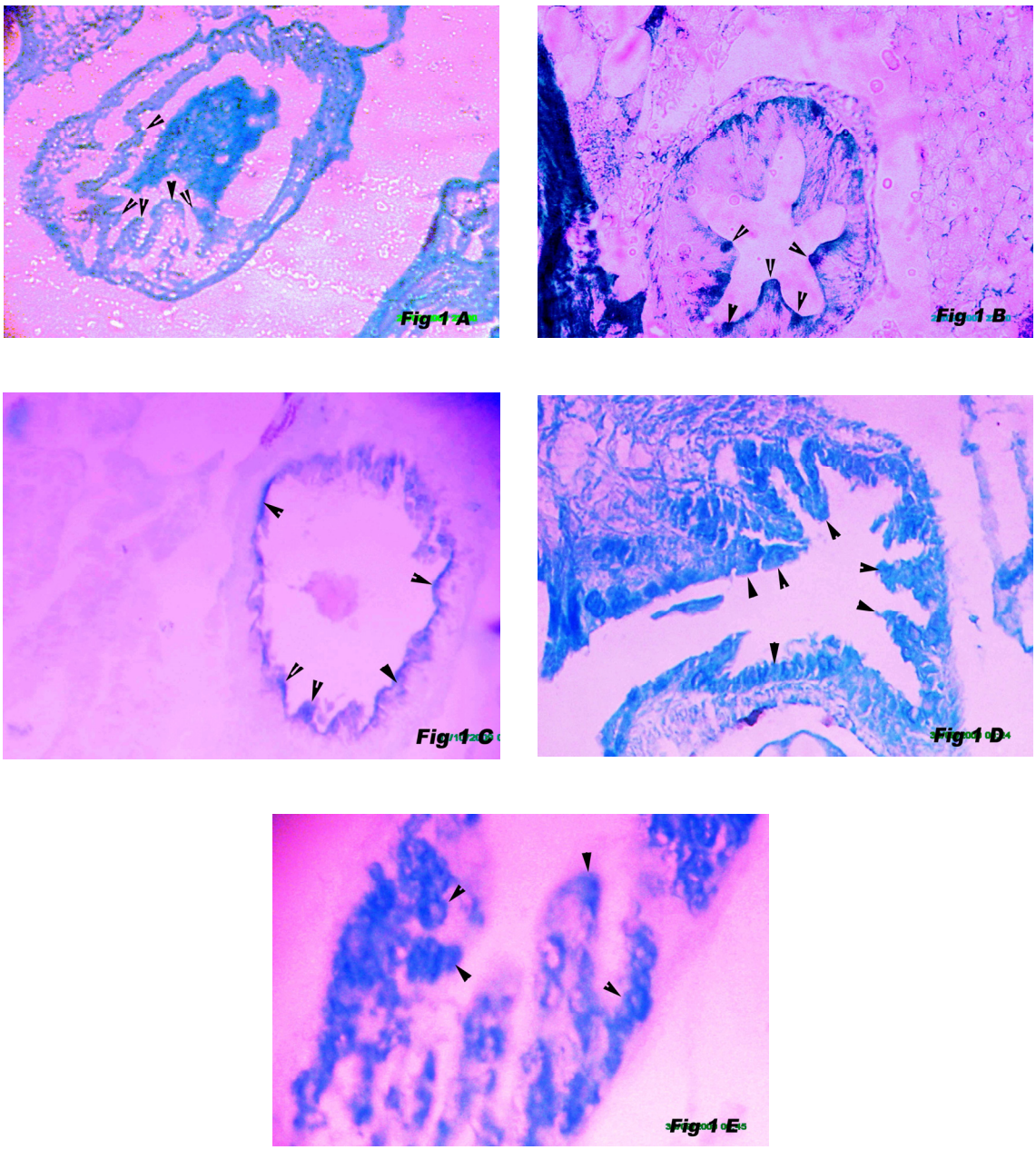

Figure 1(A-E) : Part of buccal cavity of larva two days post hatching (dph) stained with Alcian blue-periodic acid –Schiff reagent (AB-PAS) (A) untreated larva, the mucous cells are few in number and small in size (arrows) x 250 (B&D) larva treated with high dose of thyroxin (0.1ppm) or (0.1ppm+75mg/kmg methyl testosterone (MT) and cortisol (0.1ppm) the mucous cells are numerous and most of them are strongly stained with AB(blue)(arrow) x 250. (C&E) larva treated with low dose of thyroxin (0.05ppm) or (0.05ppm+MT) and cortisol (0.1ppm), the mucous cells are moderate in number and few of them are strongly stained with AB (arrows) x 250

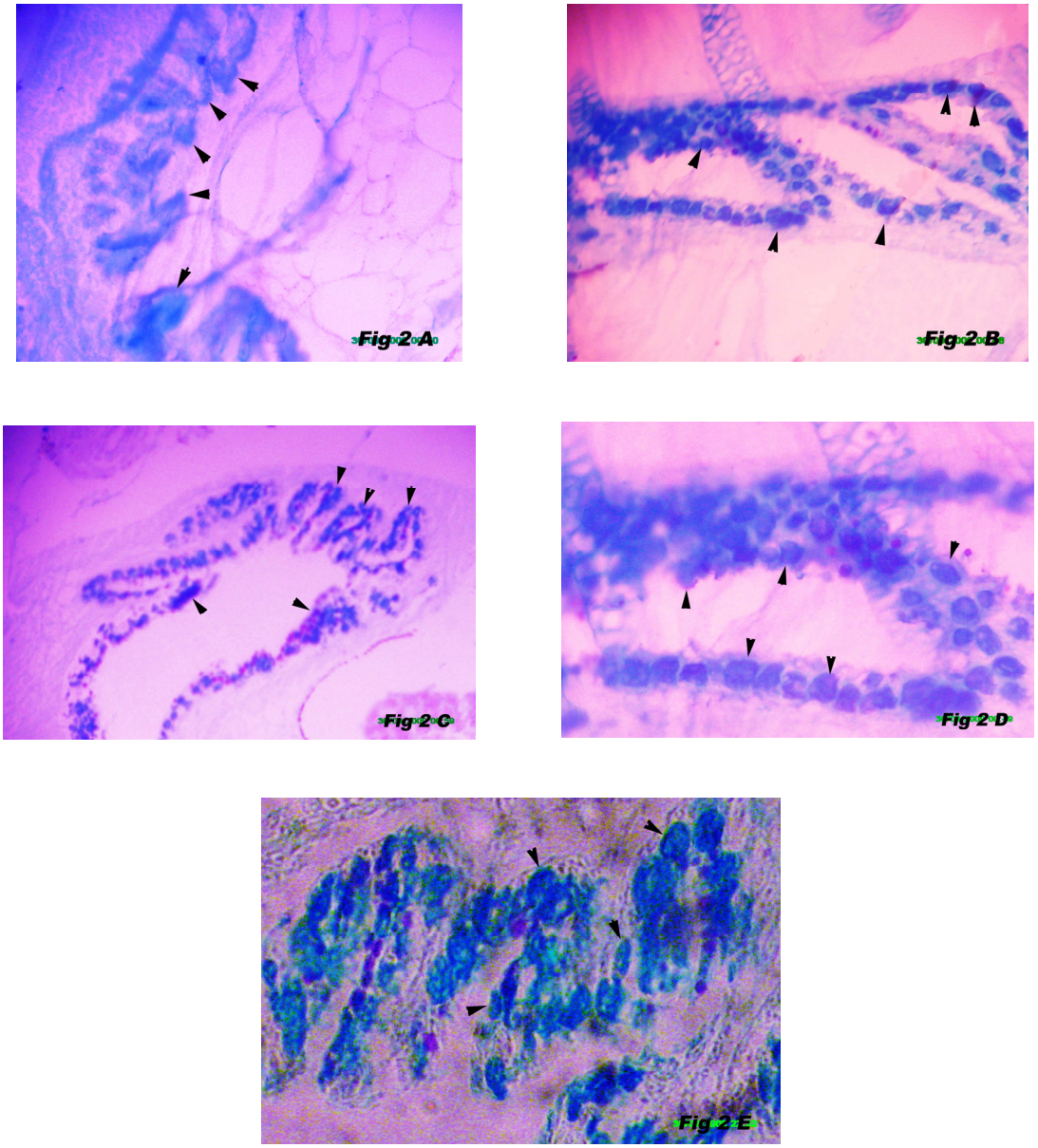

Figure 2 (A-E). Part of sagittal section of *O. niloticus* larvae 4 dph , stained with AB –PAS (A) Digestive tract of untreated larva, the mucous cells are few tract of untreated larva, the mucous cells are few in number and small in size (arrows) x 250. (B&D) Digestive tract of larva treated with high dose of thyroxin (0.1ppm) or (0.1ppm+MT) and cortisol (0.1ppm) the mucous cells have goblet shape are numerous and stained positively with AB-PAS (arrows) x 250.(C&E) Digestive tract of larva treated with low dose of thyroxin (0.05ppm) or (0.05ppm+MT) and cortisol (0.1ppm), the mucous cells are moderate in number and staining affinity (arrows) x 400.

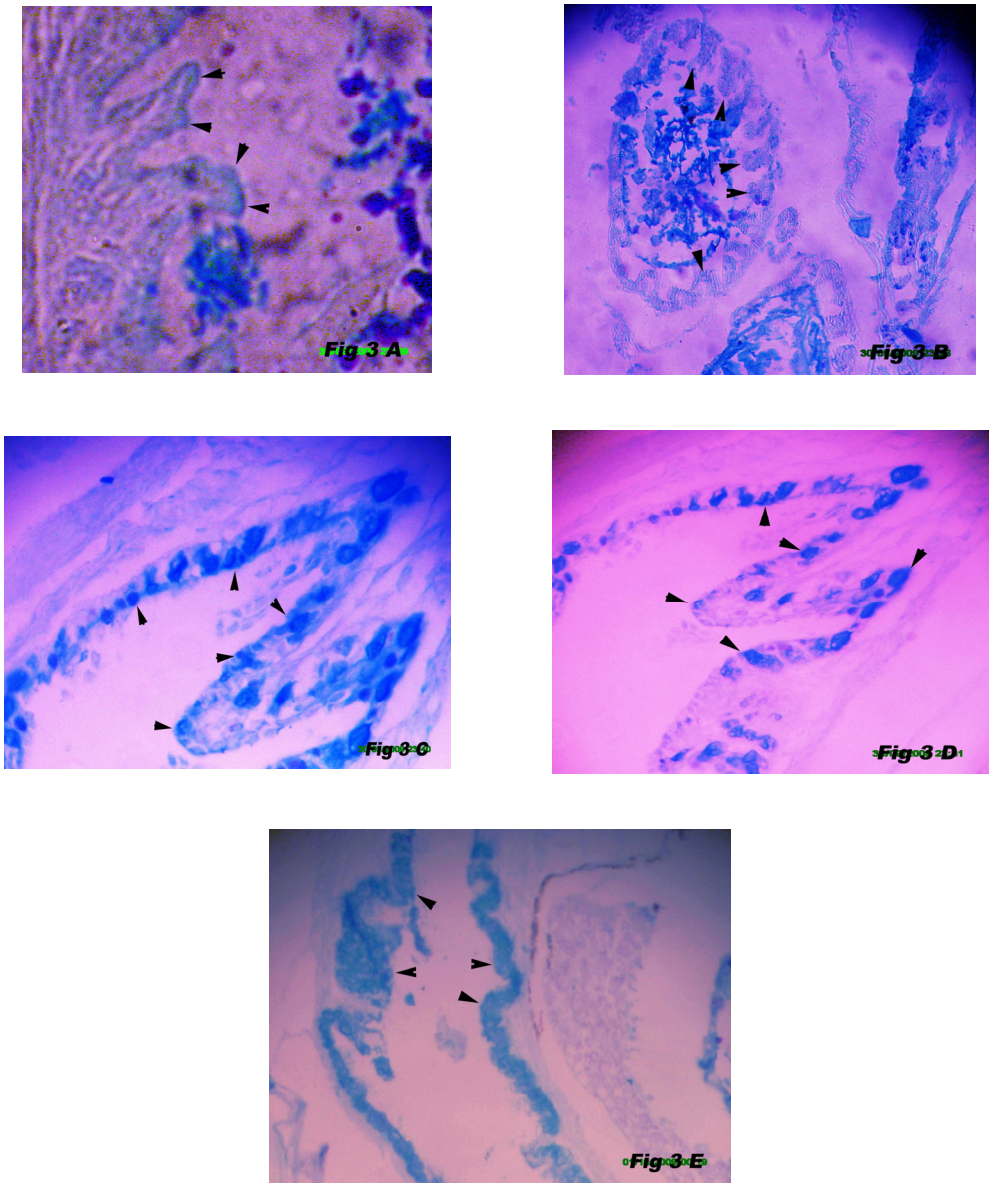

Figure 3 (A-E). Sagittal section of *O.niloticus* larvae 15 dph showing, Digestive tract (A) untreated, mucous cells are few in number (arrows) x 250.(B&D) treated with high dose of thyroxin (0.1ppm) or (0.1ppm+MT) and cortisol (0.1ppm) the mucous cells are numerous with mucousubstances (arrows) x 250. (C&E) treated with low dose (0.05ppm) or (0.05ppm+MT) and cortisol (0.1ppm). The mucous cells are moderate in number and staining affinity (arrows) x400.

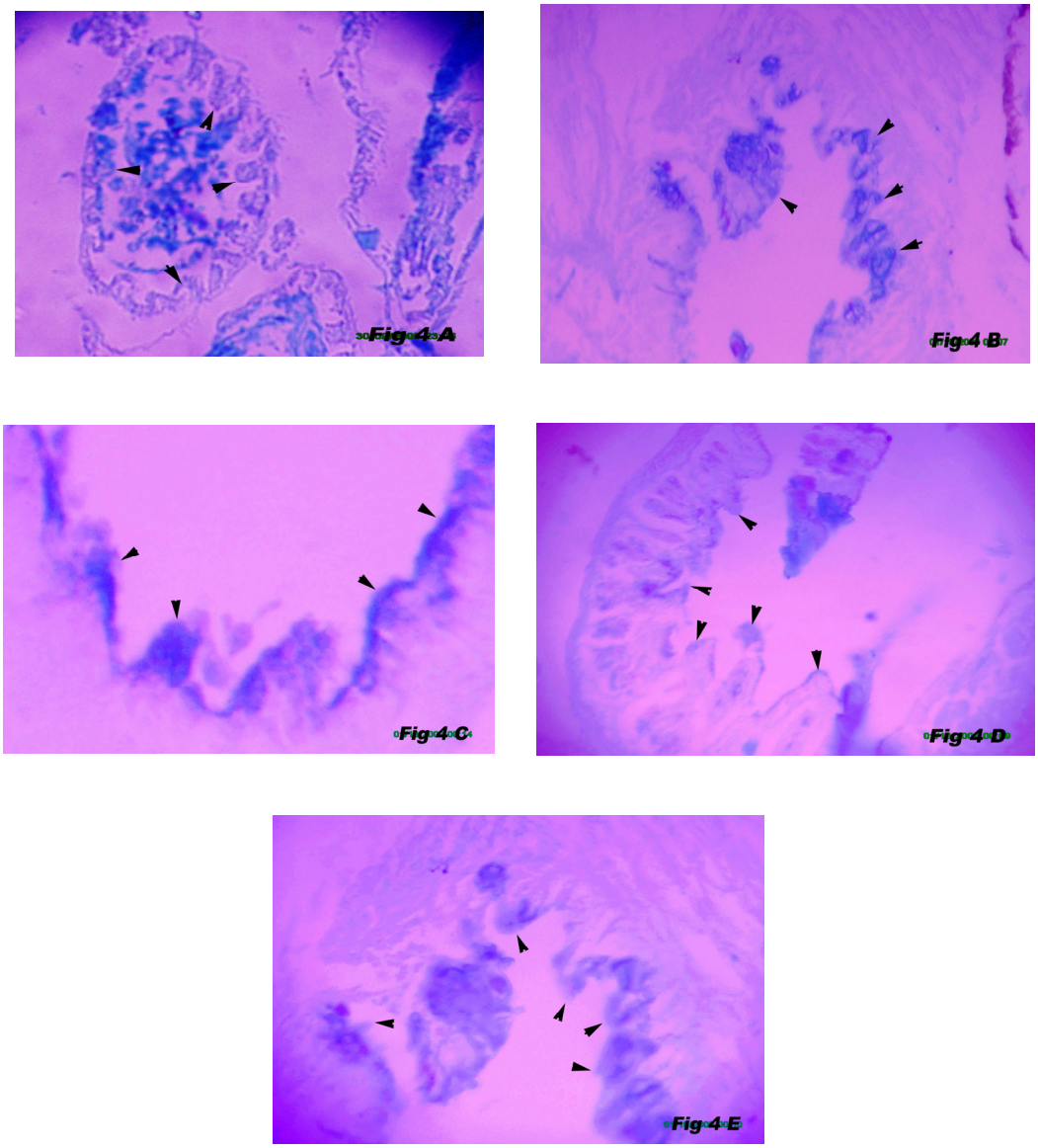

Figure 4 (A-E). Sagittal section of 22 dph *O.niloticus* larvae stained with AB-PAS, Showing, distribution of mucous cell activity in digestive tract (A) control group with small number (B&D) high dose treated larvae, the mucous cells have goblet shape, they are numerous and stained positively with AB (arrows). (C&E) low dose treated larvae. Note, mucous cells are moderate in number and mucousubstances (arrows) x 400.

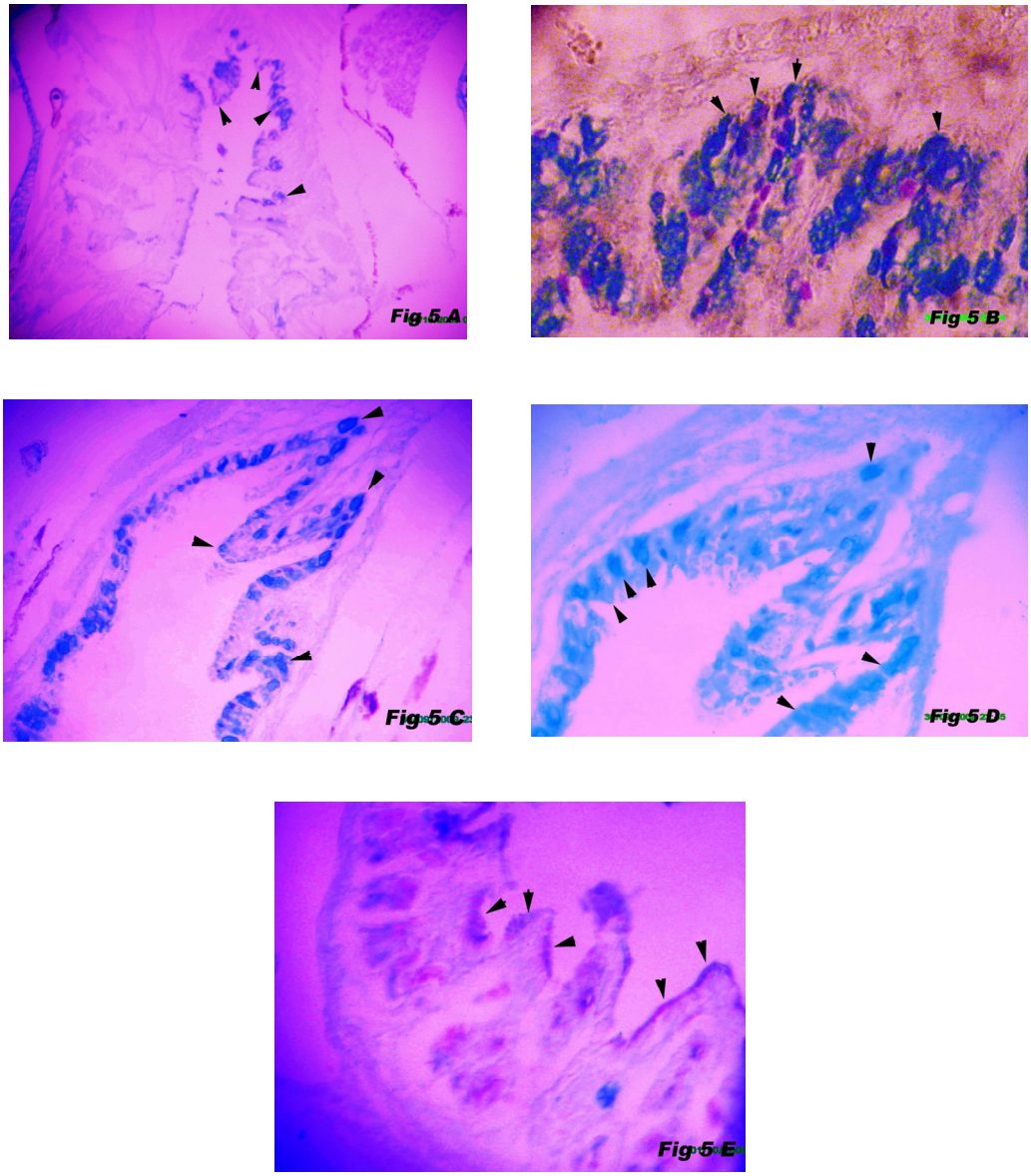

Figure 5 (A-E). Sagittal section of 32 dph *O.niloticus* stained with AB-PAS. Showing, distribution of mucous cells in the digestive tract (A) control group. Note small number of mucous cells. (B&D) High dose treated group with cortisol (0.1ppm) and thyroxin (0.1ppm) or (0.1ppm+MT). Large number of goblet and mucous cells were recorded with mucosubstances (arrows) x 250. (C&E) low dose treated group with cortisol (0.1ppm) and thyroxin (0.05ppm) or (0.05ppm+MT). Moderate number of mucous cells was noticed (arrows) x 250

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