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EFFECT OF DIETARY CALCIUM LEVELS ON GROWTH, HEALTH AND HISTOLOGICAL CHANGES OF *CLARIAS GARIEPINUS*

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Abstract

This study aimed to detect the optimal calcium requirement for *clarias gariepinus (C. gariepinus*) by feeding them with diets containing different levels of (Ca) calcium (1, 1.2, 1.4, 1.6 and 1.8 %) and illustrate their effects on growth performance, some whole body (biochemical composition and minerals), serum biochemical parameters and histological alterations. One hundred and fifty apparently healthy *C. gariepinus* with an average body weight 15.00 ± 0.50g was used. Fish were divided randomly into five equal triplicate groups (10 fish per replicate). The feed of fish was isonitrogenous, isocaloric diets in which it fed four times daily at rate of 4-5 % of body weight for 10 weeks. The results revealed that there was no significant ($P > 0.05$) in growth indices (body weight, body gain % and specific growth rate %) when compared with fish fed on control diets. There was also no significant difference ($P > 0.05$) in the average daily feed intake, feed conversion rate (FCR) and survival rates with all groups. Crude protein (CP %) in body biochemical composition showed a significant ($P < 0.05$) improvement when level of dietary inorganic-Ca is increased. Change in inorganic dietary-Ca level, had significantly affected on Ca level of whole body contents ($P < 0.05$) however the levels of the phosphorus (P), potassium (K), and magnesium (Mg) of whole body were not affected. Also the results showed that, the differences in amount of inorganic Ca in diets at the range of 1 to 1.8% could not significantly affect on growth indices, body biochemical composition, some whole body minerals and serum biochemical parameters. The histological study revealed an increasing in active

cells and melano-macrophage centers of corpuscle of stannuis of *C. gariepinus* when the dietary calcium level increased.

Key words: Calcium, Growth Performance, Health, Histological, *C. gariepinus*.

INTRODUCTION

Feeding of fish on diet contain all of essential nutrients; will grow healthy and doesn't suffer from any nutritional diseases. The highly beneficial and economically effective fish health relies on nutrition and diet formulation. Minerals are inorganic elements essential for the normal vital processes; fish can take these minerals from the diet and also from surrounded aquatic environment (Lall, 2002).

The calcium main function is a structural component of bone, exoskeleton, teeth and scales. Also, calcium is essential for blood clotting, muscle contraction, bone mineralization, proper nerve impulse transmission, and osmoregulation, maintenance of cell membrane integrity and as a cofactor for enzymatic processes (NRC, 1993). Regulation of Ca influx and efflux occurs at the gills, fins, and oral epithelia. The teleosts possess hormones with hypocalcemic action which were calcitonin, secreted by the ultimobranchial gland, and stanniocalcin (STC), secreted by the (CS) corpuscles of Stannius (Lall and Lewis-McCrea, 2007). The corpuscles of Stannius are paired organ presented in fish at posterior kidney in the anterior portion (Pruthvi raj *et al*., 2014). In marine fishes the CS cells are more active than the fresh fishes, thus indicating calcium is a factor for the activity of corpuscles of Stannius in fishes (Pruthvi raj *et al*., 2014). Exposure of fish to bad conditions such as exposure to pollution will disturb the ionic balance in fishes (Rakesh and Das, 2015). The low levels of calcium lead to poor growth and feed efficiency together with deficiency symptoms in fish.

Studies had been conducted on the effects of Ca on growth, biochemical composition and elements of whole body in fish were studied in many researches (Fontagné *et al*., 2009; Albrektsen *et al*. 2009; Kousoulaki *et al*. 2010]. Dietary Ca supplementation up to a certain level had highly beneficial effect on the performance of blue tilapia reared in Ca-free water (Robinson *et al*., 1987) but also scorpion fish fingerling reared in sea water (Hossain and

Furuichi, 2000). The dietary Ca supplementation positive effect was also studied in American cichlid (Chavez-Sanchez *et al*., 2000) and in Atlantic salmon when dietary phosphorus level was unbalanced (Vielma and Lall, 1998) and in red sea bream at high dietary P levels (Sakamoto and Yone, 1973). However, excess dietary Ca has been reported to induce bad effects in other fish species (Vielma and Lall, 1998). For these reasons, the present study aimed to illustrate the impact of various levels of calcium in the diet on the growth performance, some whole body (biochemical composition and minerals), serum biochemical parameters and the histological status of *C. gariepinus*.

MATERIALS AND METHODS

Experimental fish:

A total number of 150 live apparently healthy *C. gariepinus* with an average body weight 15±0.50 g obtained from Abassa Fish Hatchery at Sharkia province. Glass aquaria (80 X 60 X 30 cm) filled with 90 L., de-chlorinated fresh water and aerators were used to keep Fish during experiment. The water temperature, dissolved oxygen, pH, ammonia (NH3) and nitrite were measured and found to be 27 ± 2°C, 5.4 mg/l, 7.2, 0.20 mg/l and 0.02 mg/l respectively. Fish were divided into 5 equal groups (A, B, C, D and E) in which fish feed calcium by a dose of 1, 1.2, 1.4, 1.6 and 1.8 % respectively. Each group was divided into 3 replicates. Each replicate contain 10 fish. The fish were acclimated to the experimental conditions for two weeks before the start of the experiment.

Fish diets and feeding:

The basal diets contained different level of calcium at 1, 1.2, 1.4, 1.6 and 1.8 %. All fish were fed their respective diets at a level of 4-5% of body weight four times daily for 10 weeks. Feedstuffs used in diets formulation were analyzed for moisture, crude protein, ether extract and crude fiber according to the standard procedures of the A.O.A.C (1990). Isocaloric and isonitrogenous diets were prepared at Fish Research Center, Faculty of Veterinary Medicine, Zagazige University, Egypt. It contained 2900 kcal/kg ME and 30.00% CP in the form of dry pellets and was formulated to meet the nutrient requirements of

C. gariepinus has set by (NRC, 1993) and shown in Table 1. The analysed values were in close agreement with the calculated values.

Growth performance parameters:

The fish were weighed at the start and the end of the experiment. Average body weight was calculated by dividing the total weight of fish by the number of fish in each group. Body gain (BG) and feed conversion ratio (Siddiqui *et al.*, 1988). Body gain percent (BG %) (Jauncay and Ross, 1982) and specific growth rate % (SGR %) (Siddiqui *et al*., 1988) were determined.

Condition factor:

The condition factor was calculated according to Gjedrem and Gunnes (1978).

Health condition:

For evaluation of health condition of the fish during the period of the experiment, escape, defensive, tail and ocular reflexes were regularly observed according to Lucky (1977). Fish of all groups were regular observed daily for abnormal behaviours and mortality rate.

Samplings and chemical analyses:

In the first, five fish from each tank were collected and kept frozen at -20°C for later whole body composition and mineral analyses. In the final sampling, five fish from each tank were stored for whole body composition and mineral analyses. The survival of fish was calculated from daily mortality and from the final number of the surviving fish recorded in each aquarium. Proximate composition of diets and whole body composition was determined after mixing and homogenized the whole of sampled diets and fish body with mixture were analysed for crude protein (CP), crude lipid, moisture and ash; results expressed as percentage of live weight, according to the standard procedures of the A.O.A.C (1990). Cacium (Ca), phosphorus (P), potassium (K) and magnesium (Mg) were determined by atomic absorption spectrometry. Transamnases (GOT and GPT) were determined in tested fishes as described by Wooten (1964) and Oser (1965), respectively.

Table 1: Chemical composition of the experimental diets.

Ingredient	Experimental diets				
	Calcium in diets				
	A	B	C	D	E
Yellow corn	34.99	34.98	34.97	34.50	34.00
Wheat flour	10.00	10.00	10.00	10.00	10.00
Soybean meal	10.00	10.00	10.00	10.00	10.00
Fish meal	18.00	18.00	18.00	18.00	18.00
Poultry by-product meal	16.00	16.00	16.00	16.00	16.00
Vegetable oil	5.50	5.50	5.50	5.50	5.50
Vitamins and Minerals mixture*	1.50	1.50	1.50	1.50	1.50
α-cellulose	2.00	1.50	1.00	0.50	-
Calcium carbonate	-	0.50	1.00	1.50	2.00
	Calculated composition				
DM, %	82.44	82.44	82.44	82.44	82.44
CP, %	30.07	30.07	30.07	30.07	30.07
EE, %	9.80	9.80	9.80	9.80	9.80
CF, %	2.55	2.55	2.55	2.55	2.55
Ash, %	6.66	6.66	6.66	6.66	6.66
NFE, %	38.65	38.65	38.65	38.65	38.65
DE, Kcal/ kg diet**	2874.23	2874.23	2874.23	2874.23	2874.23
Ca, %	1.04	1.22	1.41	1.60	1.80
P, %	0.83	0.83	0.83	0.83	0.83
K, %	0.97	0.97	0.97	0.97	0.97
Mg, %	0.17	0.17	0.17	0.17	0.17

* Vitamin and Mineral mixture (alfakema):- Each 1 kg contains:- Vit. A 580000 I.U, vit. D3 8600 I.U, vit. E. 720 mg, vit. K3 142 mg, vit. C 0.1 mg, vit. B1 58 mg, vit. B2 34 mg, vit. B6 34 mg , vit. B12 58 mg, Folic acid 86 mg , Pantothenic acid 8 mg, Manganese sulfate 65 mg, Zinc methionine 3000 mg , Iron sulfate 2000 mg, Copper sulfate 3400 mg, Cobalt sulfate 572 mg, Sodium selenite 25 mg, Calcium iodide 25 mg and Calcium carbonate (Carrier substance) till 1000 gm.

** digestible energy calculation based on values of protein 3.5 kcal/gm, fat 8.1 kcal/gm and NFE 2.5 kcal/gm **(Santiago et al. 1982).**

(DM= Dry matter, CP= Crude protein, EE= Ether extract, CF= Crude fiber and NFE= Nitrogen free extract).

Histological changes:

Corpuscle of stannius was fixed then paraffin sections and stained with H and E routine stain (Luna, 1968).

Statistical analysis:

The obtained data in this study were statistically analyzed for variance ANOVA, LSD (Least significant difference) according to (Snedecor and Cochran, 1982). Differences among treatment means were compared using Duncan's multiple range tests (Duncan, 1995). Data were presented as mean ± SE and significance was declared at ($P < 0.05$).

RESULTS and DISCUSSION

Growth performance:

The growth performance of fish fed the experimental diets is presented in Table 2. The results showed that the feeding of fish diet contain Ca were not significantly ($P > 0.05$) affected the total final body weight (BW), BG % and SGR %, when compared with fish fed on control basal diets. Also, same table demonstrated that the average daily feed intake and total FCR were not significantly ($P > 0.05$) different with all groups. Fish fed on diet contained 1.4% calcium achieved the highest final average BW followed by fish groups fed on diet contained 1.6% calcium, while the lowest values were obtained in fish group fed on diets contained 1.2, 1.8% and control group contained 1% Ca. Regarding the SGR %, BG %, and FCR followed a same tendency.

These results clearly showed that, increasing the inorganic dietary-Ca (between 1-1.8%) could not significantly ($P>0.05$) effect on some growth indices. These results are also in accordance with Kalantarian *et al*. (2013) who suggested that changes in amount of inorganic Ca in diets at the range of 0.95-1.61% could not significantly affect the growth indices in rainbow trout fingerlings in a culture system. Also, Skonberg *et al*. (1997**)** shown that dietary P or Ca level were not affected growth, so the requirement for P is lower for growth than maximum P deposition in rainbow trout fry. *C. gariepinus* and

tilapia reared in calcium-free water showed requirement of 0.45% and 0.7% Ca in the diet, respectively.

Health status and blood biochemical parameters:

Condition factor and survival rate is shown in Table 3. It was shown that, increasing the inorganic dietary-Ca had no significant effect ($P > 0.05$) on both of condition factors and survival rate of the fish.

The level of AST and ALT in serum hadn't affected by fish fed on experimental diets as shown in Table 4. This result is coordinated with that mentioned by Hassaan *et al*. (2013) who reported that there is no significant effect in serum aspartate aminotransferase (AST) and alanine aminotransferase ALT of fish fed with Ca/P ratio diets.

There was no significant difference in serum calcium level between groups B, C, D and E; however there is a significant variation between group A and other groups as shown in Table 4. It could be explained as the strict control of ionic calcium means that the calcemic regulation system must be capable of react rapidly on various external calcium availability as supported by Abbink *et al*. (2004). Nearly similar results were obtained by Johnson (1972) who mentioned that, in the striped mullet; no differences in plasma calcium levels were observed between fish adapted to freshwater or seawater habitats. In contrast, plasma calcium concentrations in trout (Meats *et al*., 1978) and tilapia (Urasa and Wendelaar Bonga, 1987) differed significantly between fish adapted to high or low levels of calcium in the water. Hanssen *et al*. (1992) Illustrated that the effects on plasma ionic and total calcium concentrations were not related to the calcium concentration of the water after long-term acclimation.

There was non-significant ($P > 0.05$) difference in total serum protein between groups B, C, D and E and significantly increased in group A as demonstrated in Table 4. These results disagree with that of Hassaan *et al*. (2014) who stated that fish fed diet supplemented with calcium had significantly higher levels of total serum protein.

Fish biochemical composition:

Effect of dietary Ca levels on fish biochemical composition are shown in Table 5. Statistical analysis of data revealed that, small variation were showed with increasing the inorganic dietary-Ca levels, and was only significantly ($P < 0.05$) increased on whole body CP % in all treatments if compared to the control. there were no significant effect of inorganic dietary-Ca on body dry matter (DM %), body lipid content (EE %) and body ash content (Ash %) if compared to the control.

These results were supported by Kalantarian *et al*. (2013) who reported that increasing the inorganic dietary-Ca, causing increase the crude protein in whole body of fish. On the other hand, there is not dietary-Ca impact on fish protein content or protein utilization in haddock and Atlantic salmon (Albrektsen *et al*., 2009); however P inadequacy caused a decrease in the whole body protein content (Roy and Lall, 2003). Increasing body protein content with increasing the dietary-Ca could be explained that increased activity of Ca regulatory proteins such as Calamodulin and Troponin C Kalantarian *et al.*, 2013) or increasing the activity of phagocytosic cells as a result of role of Ca ion on immunological activity (Nikapitiya *et al*., 2010).

Increasing the inorganic dietary-Ca weren't significant effect between treatment on body dry matter, lipid and ash contents. Not significantly reduction for whole body fat content with increasing the dietary-Ca could be explained that the dietary Ca can lower the net absorption of dietary fat by its precipitating in the digestive tract resulting in the increased fat excretion in feces (Lorenzen *et al*., 2007). Similar results were previously reported by (Shiau and Tseng, 2007) suggested that the dietary Ca supplementation had not a significant effect on body ash levels in cod. In contrast in other fish species an increase in levels of dietary Ca or P had a positive effect on vertebrae ash levels (Chavez-Sanchez *et al*., 2000 and Vielma *et al*., 2002) especially when dietary P levels were sufficient.

Fish whole body minerals:

Effect of dietary calcium levels on fish whole body minerals are shown in Table 6. The results indicated that, change in inorganic dietary-Ca had significantly ($P < 0.05$) affected on Ca of whole body contents and not significantly ($P > 0.05$) affected the P, K and Mg of whole body contents were noticed between treatment.

Changes in the levels of Ca affect the availability of other minerals in fish (Shearer *et al*., 1994) probably due to competitive inhibition of these cations during intestinal absorption (Roy and Lall, 2003). On the other hand, dietary Ca deficiency didn't affect whole-body composition (Fontagné *et al*., 2009) and in marine fish (Hossain and Furuichi, 2000) probably due to Ca absorption from seawater was sufficient for maintaining normal tissue Ca but not for normal growth, similar results recorded in red lip mullet, giant croaker and tiger puffer.

Excess dietary Ca inhibited P absorption may be prevented by its combination with Ca to form biologically unavailable calcium phosphates in tilapia (Cowey and Sargent, 1979) and common carp (Nakamura, 1982). In addition potential dietary essentiality, dietary Ca may affect the other essential dietary minerals such as P, K and Mg (Vielma and Lall, 1998). High level of dietary-Ca interferes with the abs orption and retention of certain trace elements as K and Mg (Lall, 2002). Dietary-Ca had prevent Mg deposition both of scales and vertebrae were been found in Atlantic salmon who mentioned that calcium is a reason for the activity of corpuscles of Stannius in (Vielma and Lall, 1998) or not necessary for Mg deposition probably due to fish are able to take up Mg from the water (Kalantarian *et al*., 2013).

Histological status of *C. gariepinus* fed on calcium supplemented diets:

Morphologically:

Corpuscle stannius are paired endocrine glands. They are located on lateral sides of the head in catfish; they are reddish- brown in color. Corpuscle stannius are paired endocrine glands. They are located on lateral sides of the head in catfish, this result in opposite side to De Smet (1962) who reported that

Stannius corpuscles are tiny endocrine glands associated with the kidney of holostean and teleostean fishes. They are located in the dorsocaudal part of the trunk kidney. Also, Pruthvi raj *et al.* (2014) recorded that Corpuscle stannius is paired organ embedded in the anterior portion of the posterior kidney in fish.

Histologically:

In group A, corpuscle stannius cells are more dispersed, separated in irregular ways around blood vessels (Figs. 1, 2). In group B, corpuscle stannius cells aren't organized in regular manner, melano-macrophage centers were appeared yellow to brown in colour (Fig. 3), 1-2 active cells and lymphocytic infiltrations were appeared (Fig. 4). In group C, corpuscle stannius cells are closely packed, crowded and organized in regular manner and increased number of active cells (Figs. 5, 6). In group D, corpuscle stannius cells were appeared more organized in regular manner like rays around blood vessels, more lymphocytic infiltration, melano-macrophage centres and active cells (Figs.7, 8,9). In group E, corpuscle stannius cells were appeared more and more organized in regular manner around blood vessels, high number of active cells and melano-macrophage centers (Figs. 10, 11, 12).

With increasing calcium level, there is an increasing in active cells and melano-macrophage centers, this result in accordance with Pruthvi raj *et al*. (2014) who reported that calcium is a factor for the activity of corpuscles of Stannius in fishes, in addition that calcium and copper content of the medium plays significant role in stimulating and activating corpuscle stannius cells in fresh water fish. Corpuscle stannius cells in (groups C and E) are arranged closely packed, organized in regular manner and arranged closely packed in four phases of reproductive cycle.

CONCLUSIONS

It could be concluded that inorganic dietary-Ca (between 1-1.8%) had no clear significant ($P > 0.05$) effects on growth, biochemical composition, blood biochemical parameters, whole body minerals which is in agreement with the

generally accepted view that most of fish can absorb Ca from the surrounding environment or from diet to meet their requirements. We suggest that the optimum percent of inorganic dietary-Ca for *C. gariepinus* growth is 1.4%. The histological changes elucidate a positive relation between the increasing levels of calcium in diet and active cell of *C. gariepinus* corpuscle of stannius ensuring that the CS are stimulated to secrete stanniocalcin in order to regulate the calcium level in blood.

Table 2. Effect of dietary calcium levels on growth performance of *C. gariepinus*.

Parameters	Experimental diets				
	Calcium in diets				
	A	**B**	**C**	**D**	**E**
Initial body weight (g)	15.72 ±0.44	15.69 ±0.36	15.73 ±0.45	15.71 ±0.37	15.69 ±0.36
Final body weight (g)	19.11 ±0.62	19.10 ±0.54	19.24 ±0.52	19.21 ±0.45	19.10 ±0.48
Body weight gain (%)	21.55 ±0.50	21.75 ±1.32	22.32 ±0.58	22.35 ±0.73	21.72 ±0.53
Specific growth rate %	0.26 ±0.005	0.26 ±0.014	0.27 ±0.005	0.27 ±0.008	0.26 ±0.005
Feed consumption (g)	20.47 ±1.69	20.58 ±1.65	20.31 ±1.36	20.46 ±1.64	20.25 ±1.35
Feed conversion ratio	6.01 ±0.17	6.02 ±0.12	5.77 ±0.24	5.81 ±0.29	5.92 ±0.15

Table 3. Effect of dietary calcium levels on condition factor and survival rate of *C. gariepinus.*

Parameters	Experimental diets				
	Calcium in diets				
	A	**B**	**C**	**D**	**E**
Initial body length (cm)	10.2	10.4	10.5	10.66	10.22
Final body length (cm)	11.12	11.25	11.12	11.25	11.45
Condition factor (K)	1.39	1.34	1.4	1.35	1.27
Survival rate %	85.55 ±2.00	85.00 ±2.54	86.66 ±2.54	85.00 ±1.94	`87.22 ±3.09

Table 4. Effect of dietary calcium levels on some biochemical parameters in the blood of *C. gariepinus*.

Parameters	Experimental diets				
	Calcium in diets				
	A	**B**	**C**	**D**	**E**
AST (µmol/ml)	1.25±0.09	1.33±0.06	1.33±0.06	1.22±0.12	1.20±0.18
ALT (µmol/ml)	1.54±0.12	1.55±0.12	1.58±0.13	1.44±0.13	1.49±0.09
Serum Ca level (mg/dl)	11.6 ± 0.12^{b}	13.1 ± 0.14^{a}	13.4 ± 0.08^{a}	13.6 ± 0.11^{a}	13.9 ± 0.12^{a}
Total protein (gm/dl)	3.45 ± 0.03^{b}	4.81 ± 0.07^{a}	4.84 ± 0.03^{a}	4.91 ± 0.04^{a}	4.93 ± 0.08^{a}

[ab] Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 5. Effect of dietary calcium levels on whole body biochemical composition (% wet weight) of *C. gariepinus*.

Parameters	Experimental diets				
	Calcium in diets				
	A	**B**	**C**	**D**	**E**
Moisture (%)	70.44±1.18	70.33±0.99	70.32±0.83	70.07±0.81	69.88±0.67
Dry matter (%)	29.56±1.18	29.74±0.99	29.67±0.83	29.92±0.81	30.11±0.67
Crude protein (%)	14.66±0.70[b]	18.45±0.51[a]	18.87±0.61[a]	19.18±0.55[a]	19.57±0.44[a]
Ether extract (%)	3.37±0.42	2.73±0.45	2.48±0.31	2.52±0.34	2.38±0.37
Ash (%)	3.62±0.41	4.23±0.51	4.45±0.53	3.37±0.52	4.58±0.60
Carbohydrate (%)	7.90±2.72	4.33±2.43	3.86±2.19	3.84±2.12	3.57±1.91

[ab] Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

Table 6. Effect of dietary calcium levels on whole body mineral composition of *C. gariepinus*.

Parameters	Experimental diets				
	Calcium in diets				
	A	**B**	**C**	**D**	**E**
Calcium (%)	2.781±0.22[b]	3.47±0.32[a]	3.625±0.48[a]	3.768±0.67[a]	4.028±0.57[a]
Phosphorus (%)	1.707±0.31	2.340±0.20	2.433±0.26	2.327±0.22	2.291±0.24
Potassium (%)	0.840±0.05	0.836±0.05	0.854±0.05	0.877±0.06	0.866±0.03
Magnesium (%)	0.195±0.01	0.223±0.03	0.228±0.03	0.224±0.02	0.238±0.03

[ab] Mean in the same row with different superscripts are significantly different at ($P < 0.05$).

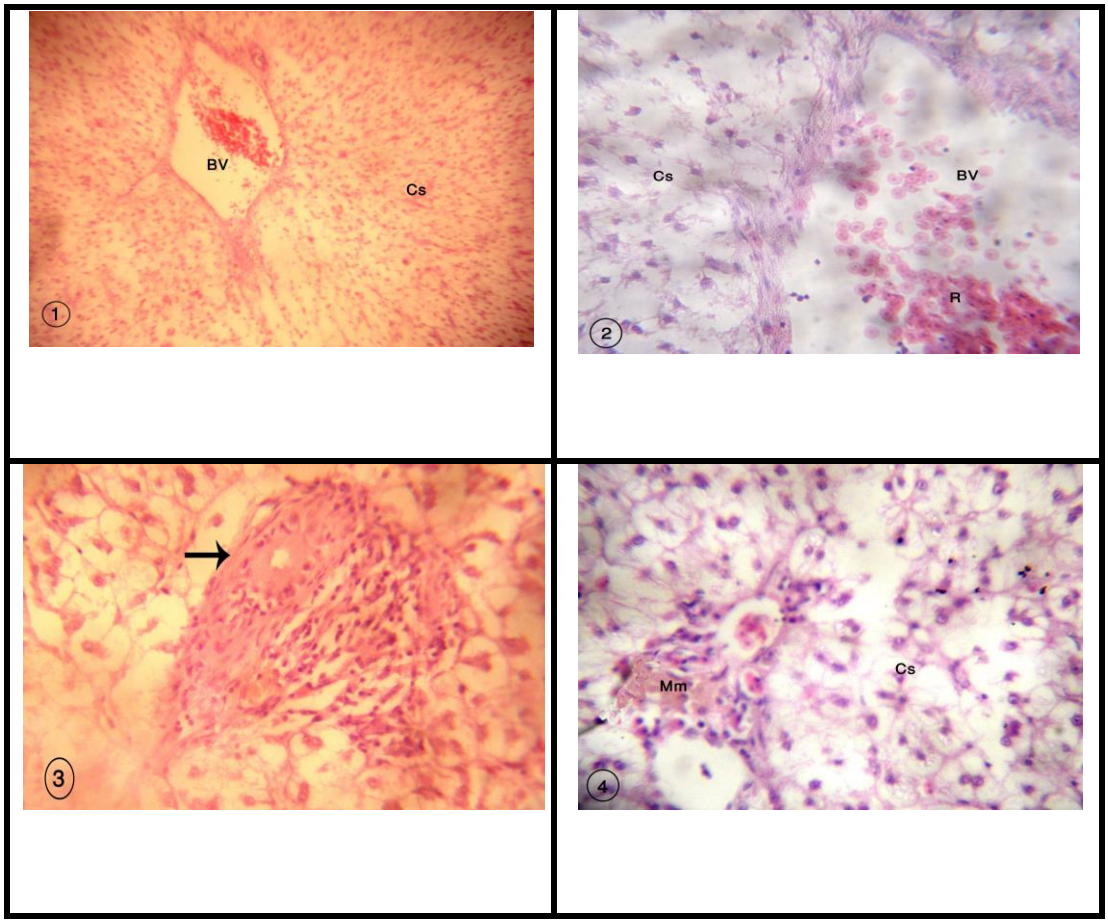

Fig. 1. A photomicrograph of control group in *C. gariepinus* showing more dispersed and separated cells of corpuscles stannius (CS), blood vessel (BV) containing RBCs, (x 100, H & E).

Fig. 2. A photomicrograph of control group in *C. gariepinus* showing more dispersed and separated cells of corpuscles stannius (CS), blood vessel (BV) containing RBCs (R), (x 400, H & E).

Fig. 3. Aphotomicrograph of 1.2% of calcium level group in *C. gariepinus* showing lymphocytic infiltrations (arrow), (x 400, H &E).

Fig. 4. Aphotomicrograph of 1.2% of calcium level group in *C. gariepinus* showing melano-macrophage centers which appeared yellow to brown in color (Mm) in between corpuscle stannius cells (CS), (x 400, H & E).

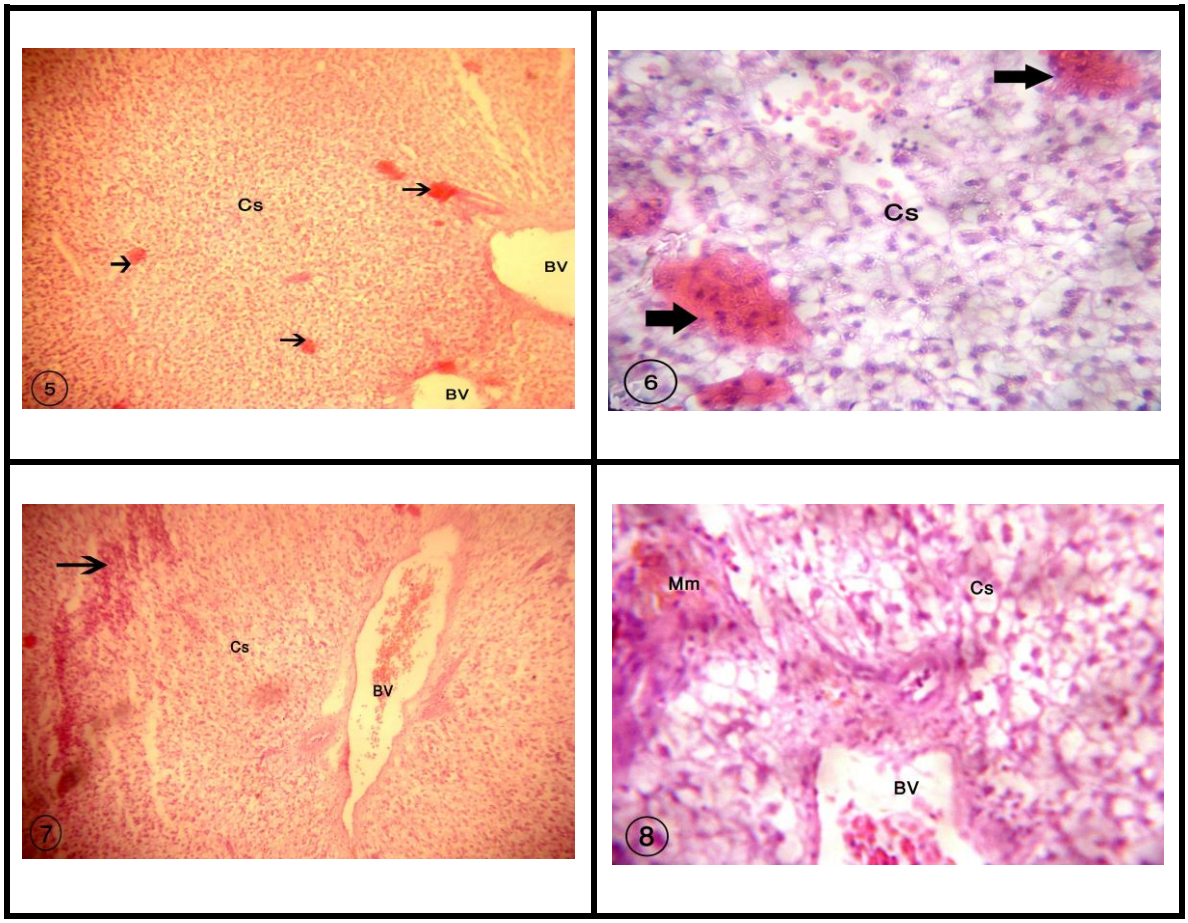

Fig. 5. A photomicrograph of 1.4% of calcium level group in *C. gariepinus* showing closely packed, crowded and organized cells of corpuscle stannius (CS), active cells (arrows), blood vessels (BV), (x 100, H & E).

Fig. 6. A photomicrograph of 1.4% of calcium level group in *C. gariepinus* showing Active cells (arrows) in between corpuscle stannius cells (CS), (x 400, H & E).

Fig. 7. A photomicrograph of 1.6% of calcium level group in *C. gariepinus* showing lymphocytic infiltrations (arrow) in between corpuscles stannius cells (CS), blood vessel (BV) containing RBCs, (x 100, H & E).

Fig. 8. A photomicrograph of 1.6% of calcium level group in *C. gariepinus* showing melano-macsrophage centers which appeared yellow to brown in color (Mm) in between corpuscle stannius cells (CS), blood vessel (BV) containing RBCs, (x 400, H & E).

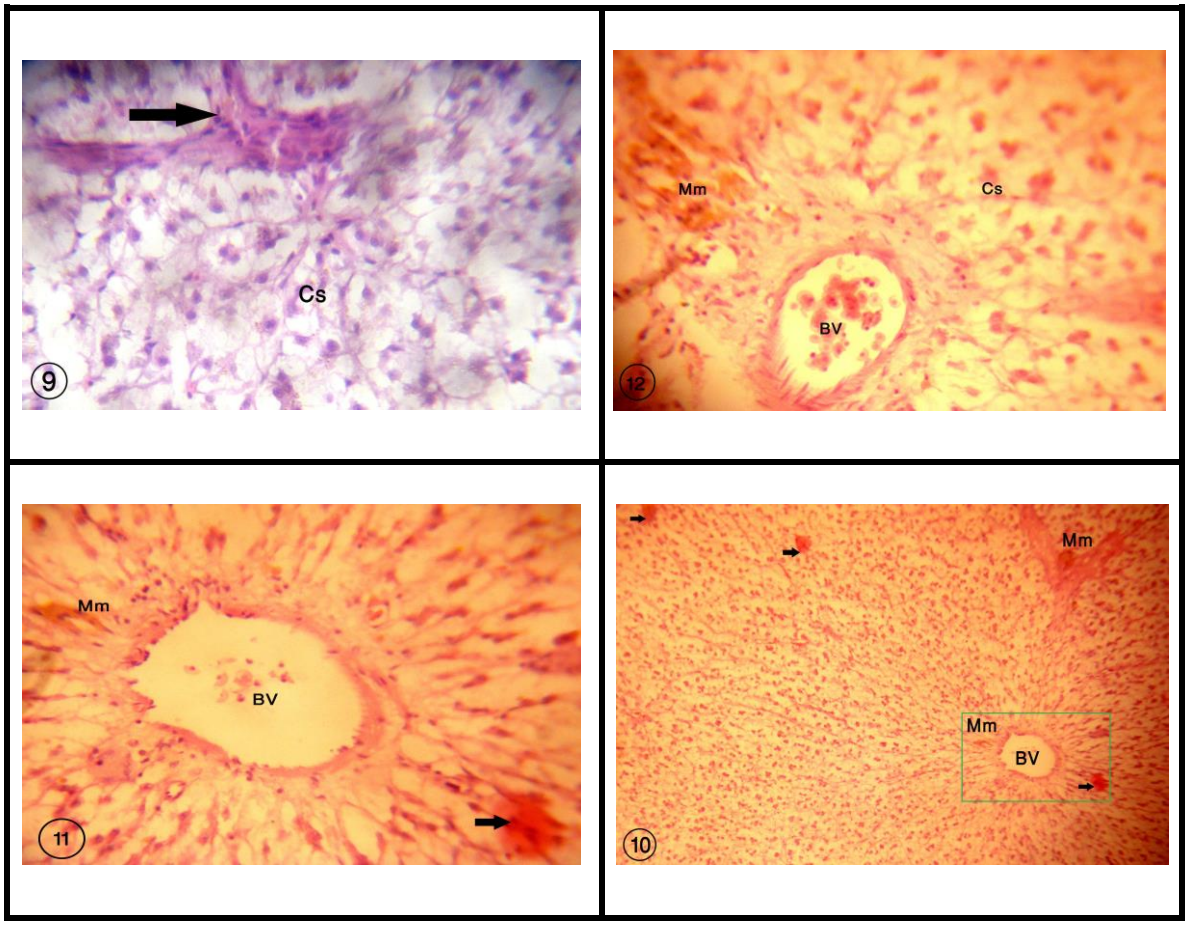

Fig. 9. A photomicrograph of 1.6% of calcium level group in *C. gariepinus* showing active cell (arrow) in between corpuscle stannius cells (Cs), (x 400, H & E).

Fig. 10. A photomicrograph of 1.8% of calcium level group in *C. gariepinus* showing high number of active cells (arrows) also melano- macrophage centers (Mm) in between corpuscle stanninus cells, blood vessel (Bv), (x 100, H & E).

Fig. 11. High magnification of illustrated rectangular area of **Fig. 10.** showing melano-macrophage centers (Mm) in between corpuscle stanninus cells(CS), active cell (arrow), blood vessel (BV), (x 400, H & E).

Fig. 12. A photomicrograph of 1.8% calcium level group in *C. gariepinus* showing more melano- macrophage centers (Mm) in between corpuscle stanninus cells (CS), blood vessel (BV), (x 400, H & E).

REFERENCE

A.O.A.C. (Association of Official Analytical Chemists), 1990. Official methods of analysis. Association of official analytical chemists. Arlington, VA, pp, 1298.

Abbink, W.; G.S. Bevelander; J. Rotllant; A.V.M. Canario and G. Flik, 2004. Calcium handling in Sparus auratus: effects of water and dietary calcium levels on mineral composition, cortisol and PTHrP levels. ournal of Experimental Biology 207, 4077-4084. Published by The Company of Biologists, 10: 1242-1254.

Albrektsen, S.; B. Hope and A. Aksnes, 2009. Phosphorous (P) deficiency due to low P availability in fishmeal produced from blue whiting (Micromesistius poutassou) in feed for under-yearling Atlantic salmon (Salmo salar) smolt. Aquaculture, 296: 318-328.

Chavez-Sanchez, C.; C.A. Martinez-Palacios; G. Martinez-Perez and L.G. Ross, 2000. Phosphorus and calcium requirements in the siet of American cichlid Cichlasoma urophthalmus (Gunther). Aguaculture Nutrition, 6: 1-9.

Cowey, C.B. and J.R. Sargent, 1979. Nutrition In Hoar WS Randall J (eds). Fish Physiology. Academic Press New York, 3: 1-69.

De Smet, W., 1962. Consideration on the Stannius corpuscles and interrenal tissue of bony fishes, especially based on researches into Amia, Acta Zool (Stockhol), 43: 201-219.

Duncan, D.B., 1995. Multiple range and multiple F-tests. Biometrics, 11, 1-42.

Fontagné, S.; N. Silva; D. Bazin; A. Ramos and P. Aguirre, 2009. Effects of dietary phosphorus and calcium level on growth and skeletal development in rainbow trout (Oncorhynchus mykiss) fry. Aquaculture, 297: 141–150.

Gjedrem, T. and K. Gunnes, 1978. Comparison of growth rate in Atlantic salmon, Pink salmon , Arctic char, Sea trout and rainbow trout under Norwegian farming condition, Aqua, (13): 135-141.

Hanssen, R.G.; J.M., Mayer-Gostan; G. Flik and S.E. Wendelaar Bonga, 1992. Influence of ambient calcium levels on stanniocalcin secretion in the European eel (*Anguilla Anguilla*). J. exp. Biol., 162: 197-208.

Hassaan, M.S.; M.A. Wafa; M.A. Soltan; A.S. Goda and N.M.A. Moghet, 2014. Effect of Dietary Organic Salts on Growth, Nutrient Digestibility, Mineral Absorption and Some Biochemical Indices of Nile Tilapia; Oreochromis niloticus L. Fingerlings. World Applied Sciences Journal, 29 (1): 47-55.

Hassaan, M.S.; M.A. Soltan; H.M. Agouz and A.M. Badr, 2013. Influences of calcium/phosphorus ratio on supplemental microbial phytase efficiency for Nile tilapia (*Oreochromis niloticus*). Egyptian Journal of Aquatic Research, 39: 205–213.

Hossain, M.A. and M. Furuichi, 2000. Essentiality of dietary calcium supplement in redlip mullet liza haemmatocheila. Aquacul. Nutr., 6: 33-38.

Jauncay, K. and B. Ross, 1982. A Guide to Tilapia Feeds and Feedings. Institute of Aquaculture, University of Stirling, Scotland, pp, 111.

Johnson, D.W., 1972. Variations in the interrenal and corpuscles of Stannius of *Mugil cephalus* from the Colorado river and its estuary. Gen. comp. Endocr., 19: 7-25

Kalantarian, S.H.; G.H. Rafiee; M. Farhangi and B. Mojazi Amiri, 2013. Effect of different levels of dietary calcium and potassium on growth indices, biochemical composition and some whole body minerals in rainbow trout (Oncorhynchus Mykiss) Fingerlings. J Aquac Res Development, 4, 3: 1-8.

Kousoulaki, K.; P.G. Fjelldal; A. Aksnes and S. Albrektsen, 2010. Growth and tissue mineralisation of Atlantic cod (Gadus Morhua) fed soluble P and Ca salts in the diet. Aquaculture, 309: 181-192.

Lall, S.P. and L.M. Lewis-McCrea, 2007. Role of nutrients in skeletal metabolism and pathology in fish-An overview. Aquaculture, 267: 3-19.

Lall, S.P., 2002. The minerals, Fish Nutrition, (3rd edn). Elsevier, Amsterdam, Netherlands, pp: 259-308.

Lorenzen, J.K.; S. Nielsen; J.J. Holst; I. Tetens and Rehfeld JF, 2007. Effect of dairy calcium or supplementary calcium intake on postprandial fat metabolism, appetite, and subsequent energy intake. Am J Clin Nutr., 85: 678-687.

Lucky, M.Z., 1977. Methods for the Diagnosis of Diseases. Ametind Publishing Co., PVT.LTD. New York, pp, 6-8.

Luna, L.G., (1968): Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology Blackiston Division McGraw-Hill Book, New York.

Meats, M.; P.M. Ingleton; I. Chester Jones; H.O. Garland and C.J. Kenyon, 1978. Fine structure of the corpuscles of Stannius of the trout, Salmo gairdneri: structural changes inresponse to increased environmental salinity and calcium ions. Gen. comp. Endocr., 36: 451-461.

Nakamura, Y., 1982. Effects of dietary phosphorus and calcium contents on the absorption of phosphorus in the digestive tract of carp. Bulletin of the Japanese Society of Scientific Fisheries, 48: 409-413.

Nikapitiya, C.; M. De Zoysa; I. Whang; S.J. Kim; C.Y. Choi, 2010. Characterization and expression analysis of EF hand domain-containing calcium-regulatory gene from disk abalone: Calcium homeostasis and its role in immunity. Fish Shellfish Immunol, 29: 334-342.

NRC (National Research Council), 1993. Nutrient Requirements of fish. National Academy Press, Washington, DC, pp, 112.

Oser, B.L., 1965. Hawk s Physiological Chemistry 14th ed. Tata McGraw Hill Publishing Co., Ltd. Bombay. New Delhi, pp, 1-1472.

Pruthvi raj, C.B.; P.B. Nagaveni and R.S. Kulkarni, 2013. Corpuscles of Stannius activity under Copper and calcium exposurein the freshwater fish, Notopterus notopterus, Int J Biol Med Res., 4 (4): 3686-3689.

Pruthvi raj, C.B.; P.B. Nagaveni and R.S. Kulkarni, 2014. Corpuscles of Stannius and serum calcium during reproductive cycle in the fish, Notopterus notopterus. International Journal of Research in Fisheries and Aquaculture, 4 (1): 63-69.

Rakesh, K.P. and V.K. Das, 2015. Dimethoate induced changes in serum Calcium and Corpuscles of Stannius in freshwater catfish Heteropneustes fossilis, after short-term and long-term exposure, American Journal of Life Sciences, 3(1): 17-21.

Robinson, E.H.; D. LaBomascus; P.B. Brown and T.L. Linton, 1987. Dietay calcium and phosphorus requriements of oreochromis aureus reared in calcium–free water. Aguaculture, 64: 267-76.

Roy, P.K. and S.P. Lall, 2003. Dietary phosphorus requirement of juvenile haddock (Melanogrammus aeglefinus L.). Aquaculture, 221: 451-468.

Sakamoto, S. and Y. Yone, 1973. Effect of dietary calcium/phosphorus ratio upon growth, feed efficiency and blood serum Ca and P level in red sea bream. Bulletin of the Japanese Society of Scientific Fisheries, 39: 343-348.

Santiago, C.B.; M. Banes-Aldaba and M.A. Laron, 1982. Dietary crude protein requirement of Tilapia nilotica fry Kalikasan, philipp. J. Biol., 11(2-3): 255-265.

Shearer, K.D.; T. Asqard; G. Andorsdottir and G.H. Aas, 1994. Whole body elemental and proximate composition of atlantic salmon (Salmo salar) during the life cycle. Fish Biology, 44: 785-797.

Shiau, S.Y. and H.C. Tseng, 2007. Dietary calcium requriements of junenile tilapia Oreochromis niloticus*O aureus reared in fresh water. Aguaculture Nutrition, 13: 298-303.

Siddiqui, A.Q.; M.S. Howlader and A.A. Adam, 1988. Effects of dietary protein levels on growth, feed conversion and protein utilization in fry and young Nile Tilapia (*Oreochromus niloticus*). Aquaculture, 70: 63-73.

Skonberg, D.I.; L. Yogev; R.W. Hardy and F.M. Dong, 1997. Metabolic response to dietary phosphorus intake in rainbow trout (Oncorhynchus mykiss). Aquaculture, 157: 11-24.

Snedecor, G.W. and W.G. Cochran, 1982. Statistical methods. 8th Ed., Ames. Lowa state university.

Urasa, F.M. and S.E. Wendelaar Bonga, 1987. Effects of calcium and phosphate on the corpuscles of Stannius of the teleost fish, *Oreochromis mossambius*. Cell Tissue Res., 249: 681-690.

Vielma, J. and S.P. Lall, 1998. Phosphorus utilization by Atlantic salmon (Salmo salar) reared in freshwater is not influenced by higher dietary calcium intake. Aquaculture, 160: 117-128.

Vielma, J.; J. Koskela and K. Ruohonen, 2002. Growth, bone mineralization, and heat and low oxygen tolerance in European whitefish (Coregonus lavaretus L.) fed with graded levels of phosphorus. Aquaculture, 212: 321-333.

Wooten, I.D.P., 1964. In: Microanalysis in medical biochemistry. J and A Churchill td., London W.1.

تأثير مستويات الكالسيوم في العليقه على النمو والصحه والتغييرات النسيجية فى القرموط الافريقي

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الملخص العربى

تم تصميم هذه الدراسة لتحديد النسبة المثلى من متطلبات الكالسيوم للقراميط عن طريق تغذيتهم على علائق تحتوي على مستويات مختلفة من الكالسيوم (١، ١.٢، ١.٤، ١.٦ و ١.٨٪) ودراسة تاثيرهم على أداء النمو، وبعض مكونات الجسم الكيميائية والمعدنية، بعض القياسات البيوكيميائية في مصل الدم والتغيرات النسيجية. تم استخدام ١٥٠ قرموط بمتوسط وزن ١٥ ± ٠.٥٠جرام. تم تقسيم القراميط عشوائيا إلى خمسة مجموعات احتوت كل منها علي ثلاث مكررات وكل مكرر يحتوي علي ١٠ قراميط. تم تغذية القراميط علي خمسة علائق متساوية في نسبة البروتين (٣٠ %) والطاقة المهضومة (٢٨٧٠ كيلوكالوري/كجم) وتقدم اربع مرات يوميا بمعدل ٥% من وزن القرموط لمدة ١٠ اسابيع.

كشفت النتائج أن اضافة مستويات مختلفة من الكالسيوم الى علائق القراميط لم تظهر اى تاثير معنوى على مؤشرات النمو (وزن القراميط ، نسبة الزيادة اليومية نسبة معدل النمو) ومتوسط استهلاك العلف ومعامل التحويل الغذائى ومعدل البقاء مقارنة بالعليقة الضابطه.

مع زيادة الكالسيوم فى علائق القراميط ادت الى زيادة معنوية فى نسبة البروتين الخام فى جسم القراميط. التغيير في مستويات الكالسيوم، أثرت بشكل معنوى كبير على محتويات الكالسيوم وتاثير غير معنوى على الفسفور والبوتاسيوم والمغنيسيوم فى جسم القراميط.

أظهرت نتائج تلك الدراسة أن التغيرات في مستويات الكالسيوم في علائق القراميط من ١–١.٨٪ لا تؤثر بشكل كبير على مؤشرات النمو ومكونات الجسم الكيميائية والمعدنية، القياسات البيوكيميائية في مصل الدم، في حين فقط اثرت بشكل معنوى كبير على نسبة البروتين الخام ونسبة الكالسيوم الخام فى جسم القراميط. واقترح أن النسبة المثلى من الكالسيوم لنمو القراميط هي ١.٤٪ كالسيوم. بالنسبة للتغيرات النسجية هناك زيادة في أعداد الخلايا النشطة مع زيادة نسبة الكالسيوم في الاكل.