Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 24(5): 539 – 560 (2020)
www.ejabf.journals.ekb.eg

Effect of the different stocking density on behavior, performance and welfare of the Nile tilapia (*Oreochromis niloticus*)

Azhar F. Abdel Fattah , Fayza A. Ahmed, Al-Sadik Y. Saleem , Hesham H. Mohammed , Mohamed I. Youssef and Enas N. Said

Department of Veterinary Public Health, Faculty of veterinary medicine, Zagazig University, Zagazig, Ash Sharqia Governorate 44519, Egypt

***Corresponding Author:** azharfakhry65@yahoo.com

ARTICLE INFO

Article History:
Received: May 12, 2020
Accepted: July 24, 2020
Online: Aug. 2020

Keywords:
Nile tilapia;
Stocking density;
Surfacing behavior;
Aggressive patterns;
Crossing test.

ABSTRACT

The current study was carried out to detect the influence of various stocking densities on behavior and the biological performances of Nile tilapia (*Oreochromis niloticus*) fingerlings. Fingerlings (30±5g) were stocked in duplicate at four stocking densities; low density 15 (group1 as control), medium density 25 (group 2) and high density such as 35 (group 3) and 45 (group 4) fish/aquarium, in glass aquaria 30×40×100 cm for 10 weeks.

The results showed that the surfacing behavior was higher in high stocking density reared groups than the low and medium density reared groups. Moreover, the aggressive behavior with all patterns was markedly higher in fish raised at high stocking density than the low and medium density raised fish. The crossing test showed that low density raised fish was more active than medium and high stocking density raised fish. Final body weight was markedly decreased with increased density, While, the fish reared at low stocking density showed a marked increase of daily weight gain (DWG). Finally, high-density culture is considered as chronic stress for Nile tilapia fingerlings due to increasing surfacing behavior and the appearance of all patterns of aggression.

INTRODUCTION

Fish culture is an essential source of fish and fish products that provides a valuable source of animal protein and important micronutrients for balanced nutrition and good health (**Srinivasan *et al.*, 2015**). There is an increasing demand on fish meat worldwide as it contains a healthy and high quality protein. So, fish culture showed a remarkable development in the last decades (**Pavanelli *et al.*, 2008**). Tilapia is the second most essential farmed fish in the world, after carps. Tilapia culture is practiced in most of the tropical, subtropical and temperate regions. Major attention has been paid to tilapia farming in recent years **(Osofero *et al*., 2009).** Tilapia is the main fish species for culture over the world. It mostly is the choice because of its rapid growth rate, easily breeding, highly bearing to environmental cues, and high market require **(El-Sayed, 2006)**. Stressors in Tilapia farming include improper water temperature, and overstock, incorrect feeding regime. Both types of stress result in a characteristic stress response **(Barton & Iwama, 1991)**. In many farmed fish species, growth is negatively associated to stocking density and this is principally attributed to social interactions **(Silva *et al*., 2000).**

The apparent potency of tilapia culture could be maximized by promoting its culture densities. The intensive culture is one of methods that have been adapted to increase tilapia production (**Salama *et al.* 2006**). However, the impaired growth and disease prevalence have been regularly determined in fish cultured at high stocking density, although, it is unknown whether these problems are the raised results of high stocking densities or the related suboptimal water parameters (**Ellis *et al.*, 2002**). The stocking density, Feeding technique and management procedures, all have potential influences on stress responses, subsequent stress tolerance, signs of health, and the occurrence of aggressive behavior (**Ashley, 2007**). The growth of fish reared at high stocking density is mainly limited by water quality (**Bjornsson, 1994).** The stocking density has major effect on growth, immunity and survival rate (**Salas-Leiton *et al.*, 2010**). Many stressors have been shown to result in alterations in fish behavior such as feeding, activity and aggression **(Schreck *et al*., 1997)**.

For this reason, the objective of this study was to assess the effect of different stocking densities on behavior and performance of Nile tilapia, *Oreochromis niloticus* fingerlings.

MATERIALS AND METHODS

This study was carried out at Fish Management and Behavior Research Unit, Department of Veterinary Public Health of Faculty of Veterinary Medicine, Zagazig University- during the period from November 2018 to February **2019**.

Fish management and water hygiene

Fish fingerlings were acclimated to aquarium water temperature to avoid stress. For handling transportation, a nylon hand net was used separately for each aquarium to avoid transmission of infection. Mortality was recorded daily and morbidity was followed up.

A total number of 240 healthy Nile tilapia fingerlings (with average body weight 30 g) were obtained from a private fish farm at Ismailia Governorate. Fish were transported to Fish Research Unit of Faculty of Veterinary Medicine (Zagazig University), where fish were acclimated for two weeks in cement pond. Then fish were transported to Fish Management and Behavior Research Unit, Department of Veterinary Public Health. Fish were divided into four duplicated aquaria (90 l/aquaria, 100 x 30 x 40 cm) under four rearing stocking density groups. 1^{st} group (G_1, control): 15 fish; 2^{nd} group (G_2): 25 fish; 3^{rd} group (G_3): 35 fish; 4^{th} group (G_4): 45 fish.

Fish have been acclimated for 10 days before starting the experiment. Each aquarium was supplied with continuous aeration; water temperature was regulated by thermostatically controlled heater and thermometer. Aquaria water was completely changed five times weekly by de-chlorinated water from water storage tank. Each aquarium contained electrical aerator and filter to remove the organic waste matter in each aquarium and source of dissolved oxygen, heater (Thermostat) to keep aquarium at optimum temperature required for *Oreochromis nilotica*, Water thermometer to measure the water temperature daily and aquarium net for fish handling and transporting. Fish were kept in the aquarium parameters according to **APHA (1998)** as shown in **Table (1)**:

Table (1). Physico-chemical parameters of water in the aquarium during the experiment

Parameters	Level
Dissolved Oxygen	6.5 ± 0.5 mg/l
Total Ammonia	< 0.02 mg/l
Nitrite	< 0.05 mg/l
pH	8 ± 0.5
Water Temperature	26 ± 2°C
Salinity	0.5 ± 0.1 ‰
Water Hardness ($CaCO_3$)	190 ± 10 mg/l

The basal diet was produced by Cairo Poultry Processing Company (CPPC). It was formulated in form of dry floated pellets, to meet the nutrient requirements of Nile tilapia fingerling. The ingredients and proximate composition of the experimental diet are presented in **Table (2)** according to **NRC (1993)**.

Table (2). Chemical composition of the diets used in the experiment

Composition	%
Fish meal, 66%	20
Soybean meal, 44%	20
DDGS, 28%	10
Yellow corn	15
Corn gluten, 62%	4.55
Rice bran	26.45
Vegetable oil	3.50
L-Lysine HCL 98%	-
D L- Methionine	-
Calcium carbonate	-
Vitamin. mineral premix*	0.50
Total	**100**
Calculated composition	
Dry matter	86.47
Crude protein	32.01
Ether extract	11.47
Crude fiber	4.27
Ash	7.60
NFE	34.06
Calcium	0.89
Phosphorus	1.19
Lysine	1.85
Methionine	0.71
**DE ** **	3007.46 **(Kcal/ kg)**

* Vitamin and mineral mixture (per kg diet) {Vit. A: 6000 I.U; D_3 2.000 I.U; E: 500mg, k_3: 12.0mg; C: 1.000mg; B_1: 10mg; B_2: 15.0mg; B_6: 7.5mg; B_{12}: 0.1mg; Biotin: 0.2mg; Folic acid: 0.4mg; choline Hcl: 1.0g; inosit: 3000mg; Pantothemic acid: 50mg; Nicotinic acid: 100mg; P-Aminobenzonic acid: 50 mg; iron: 80mg; copper: 5g; zinc: 40g; Sodium selenite: 100mg; potassium iodide: 300mg; and cobalt sulphate: 100mg}

DE: digestible energy calculated based on values of protein 3.5kcal/g; fat 8.1kcal/g; NFE 2.5 kcal/g according to **(Santiago *et al*., 1982),

Feeding was three times daily at (9:00 AM), at (1:00 PM) and at (4:00 PM) and fish was fed by hand 6 days a week. Feed was given only as much as they can eat within **(**5 min) according to (**Scheurmann, 2000**)**.** The daily amount of food was kept constant at 3% of the total wet biomass of fish, throughout the experimental time according to (**Chowdhury, 2011**)**.** It was adjusted almost every 2 weeks, when the entire population of each aquarium was weighted. Nile tilapia was identified by short plastic strips applied in dorsal fin of each fish to facilitate observation for the fish during the experimental period according to **(Khalil *et al.* 2016).**

Medication: Potassium permanganate (2 mg/l) and Oxytetracycline (50 mg/Kg b.wt.) were used to treat fish from *columnaris* disease that affects most fresh water fish during stress. NaCl (1 g/l) was used as a protection against fish disease (2) times per week after water changing.

Observation and data collection:

Behavior was recorded in the period between (09:00 am) till (04:00 pm) for 10 weeks by using focal sample technique for 45 sec/fish, Intervals during one hour daily. Visually by using a note book for recording behavior, a stop watch, multipurpose counter and video camera according to **(Altmann, 1974)**. The behavioral activities were recorded 15 min/treatment. Intervals are taken through 8 hour's weekly. The observed behavior pattern was recorded as the following:

Surfacing behavior: Mean frequency and time (sec/8 hours) of the fish rise periodically to surface to gulp air, according to **Ferey & Miller (1972).** It is piping for air near the water surface due to low dissolved oxygen in aquarium, according to **Noga (1996).**

Aggressive behavior: Refers to fighting and means the act of initiating an attack according to **Ferey & Miller (1972)** and **Fall (2005).**

- **(a) Approach:** Mean frequency and time (sec/8 hour) of the direct movement of one fish toward another fish.
- **(b) Chasing:** Mean frequency and time (sec/8 hour) swimming of one fish vigorously after another fish.
- **(c) Fin Tugging:** Mean frequency and time (sec/8 hour) of one fish bites the fin of another fish.
- **(d) Biting:** Mean frequency and time (sec/8 hour) of one fish bites with its sharp mouth any region of another fish.
- **(e) Butting:** Mean frequency and time (sec/8 hour) of one fish butts with the snout against genital papilla of another fish.
- **(f) Fleeing:** Mean frequency and time (sec/8 hour) of one fish swims away from the opponent.
- **(g) Mouth Pushing:** Mean frequency and time (sec/8 hour) of two fish standing face to face with their opened mouth against each other.
- **(h) Spreading of fins:** Mean frequency and time (sec/8 hour) of one fish expands or spreads all fins.

Number of midline crossing: The aquarium was divided by a midline externally and the numbers of midline crossing from fish through 5 minutes were detected for each aquarium according to the protocol and calculations of **Scott *et al*. (2003)**.

Live fish performance

To calculate average body weight every 15 days, all fish in each group were weighted then divided the total weight of fish by the number of fish in each group according to **Khalil *et al.* (2016)**

Data handling statistical analysis

All experiments data were collected, arranged, summarized and then analyzed using SPSS version 21 Statistical Analysis System package **(SPSS, 2012)**. Results expressed as Mean ±SD.

1- Mixed model ANOVA test was used to test behavioral parameters for different groups during consecutive weeks of experiment. Interaction plot was used to compare between means of each behavioral parameter in different groups at weeks of experiment.

2- One- way analysis of variance (ANOVA) test was applied to test differences at body weight of fish at different groups. Tukey’s honesty significant test was applied after significant results (**P. value < 0.05 was considered statistically significance**).

RESULTS

Surfacing behavior (frequency and duration):

Results in **Table (3)** showed that the effect of weeks of experiment produced insignificant difference in reading of surfacing frequency among groups (P> **0.05**). By looking to interaction plot (**Fig. 1)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest surfacing behavior recorded was in the G_2 (**0.1±0.14**), and the lowest behavior was recorded in G_4. By going on weeks of experiment, the behavior in week 5 showed an increase in all groups but G_4 was the highest. In the last week, G_2 was increased in the surfacing frequency behavior while other groups were decreased. The general look to interaction plot showed that G_1 **(0.01 ± 0.04)** had the minimum values of frequency surfacing behavior compared to the other three groups, and G_4 nearly showed the highest values (**0.51 ± 0.45**) after the first week of experiment.

Results **(Table 3)** showed that the effect of weeks of experiment significantly produced a significant difference in reading of surfacing duration among groups (P< 0.05). By looking to interaction plot **(Fig. 2)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest surfacing behavior recorded was in G_2 **(0.36±0.51)**, and the lowest behavior was recorded in G_4. By going on weeks of experiments, the behavior in week 5 showed that G_4 was the highest. In the last week, G_2 was increased while other groups were decreased. The general look to interaction plot showed that G_1 showed the minimum values **(0.06 ± 0.20)** of surfacing duration behavior compared to the other three groups, and G_4 nearly showed the highest values **(7.75 ± 8. 43)** after the first week of experiment.

Approach behavior (frequency and duration):

Results showed that the effect of weeks of experiment significantly produced a significant difference in reading of approach frequency among groups (P< **0.01**). By looking to interaction plot **(Fig. 3),** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest approach frequency behavior recorded was in G_3 **(0.37±0.33)**, and the lowest approach frequency behavior was recorded in G_4. By going on weeks of experiment, the behavior in week 5 showed a decrease in all groups. In the last week all groups showed decrease in the behavior or even remained stable. The general look to interaction plot showed that G_1 **(0.01 ± 0.04)** showed the minimum values of approach behavior compared to the other three groups, and G_3 nearly showed the highest values **(0.52 ± 0.44)** after the first week of experiment **(Table 4)**.

Results showed that the effect of weeks of experiment significantly produced a significant difference in reading of approach duration behavior among groups (P< **0.05**). By looking to interaction plot **(Fig. 4)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest approach behavior recorded was recorded in G_3 **(0.44±0.53)**, and the lowest behavior was recorded in G_4. By going on weeks of experiments, the behavior in week 5 showed a decrease in all groups. In the last week all groups showed decrease in this behavior or even remained stable. The general look to interaction plot showed that G_1 **(0.01 ± 0.04)** showed the minimum values of approach duration behavior compared to the other three groups, and G_3 nearly showed the highest values **(0.76 ± 0.88**) after the first week of experiment **(Table 4)**.

Table (3): Mean ± SD of Surfacing behavior in the four groups during ten weeks of experiment.

Weeks	Surfacing Frequency				Surfacing Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.01 ± 0.04	0.1 ± 0.14	0.04 ± 0.08	0.01 ± 0.04	0.06 ± 0.20	0.36 ± 0.51	0.24 ± 0.52	0.08 ± 0.24
2	0.11 ± 0.21	0.16 ± 0.13	0.18 ± 0.11	0.11 ± 0.16	0.36 ± 0.79	2.04 ± 2.25	1.85 ± 2.06	2.54 ± 3.95
3	0.1 ± 0.16	0.23 ± 0.19	0.12 ± 0.13	0.4 ± 0.46	0.98 ± 1.83	1.80 ± 2.13	1.79 ± 2.13	5.58 ± 6.49
4	0.18 ± 0.16	0.34 ± 0.28	0.12 ± 0.13	0.38 ± 0.43	0.98 ± 1.14	2.85 ± 3.20	1.51 ± 1.99	3.58 ± 3.94
5	0.23 ± 0.28	0.22 ± 0.12	0.45 ± 0.37	0.51 ± 0.45	2.10 ± 2.88	3.58 ± 2.71	5.94 ± 4.93	7.75 ± 8.43
6	0.11 ± 0.21	0.2 ± 0.15	0.32 ± 0.29	0.35 ± 0.21	1.33 ± 2.89	1.91 ± 1.78	4.85 ± 4.01	6.49 ± 5.34
7	0.14 ± 0.2	0.23 ± 0.17	0.25 ± 0.28	0.22 ± 0.24	0.98 ± 1.43	4.29 ± 3.80	3.89 ± 4.88	3.69 ± 4.32
8	0.19 ± 0.29	0.44 ± 0.32	0.29 ± 0.21	0.49 ± 0.4	2.15 ± 3.81	9.41 ± 8.11	5.18 ± 3.50	7.14 ± 6.31
9	0.1 ± 0.13	0.32 ± 0.28	0.25 ± 0.23	0.47 ± 0.28	0.96 ± 1.41	4.26 ± 4.21	4.70 ± 4.36	7.03 ± 5.55
10	0.06 ± 0.12	0.42 ± 0.23	0.26 ± 0.2	0.33 ± 0.3	0.98 ± 2.10	8.68 ± 5.55	5.75 ± 4.80	5.14 ± 6.39

Table (4): Mean ± SD of Approach behavior in the four groups during ten weeks of experiment.

Weeks	Approach Frequency				Approach Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.17 ± 0.19	0.11 ± 0.18	0.37 ± 0.33	0.05 ± 0.09	0.2 ± 0.24	0.16 ± 0.28	0.44 ± 0.53	0.05 ± 0.09
2	0.04 ± 0.12	0.03 ± 0.05	0.21 ± 0.21	0.15 ± 0.13	0.05 ± 0.16	0.03 ± 0.05	0.38 ± 0.4	0.17 ± 0.15
3	0.09 ± 0.24	0.33 ± 0.31	0.52 ± 0.44	0.11 ± 0.19	0.11 ± 0.31	0.39 ± 0.34	0.76 ± 0.88	0.14 ± 0.24
4	0.03 ± 0.08	0.1 ± 0.22	0.34 ± 0.32	0.15 ± 0.2	0.03 ± 0.08	0.1 ± 0.22	0.5 ± 0.77	0.23 ± 0.31
5	0 ± 0	0 ± 0	0.13 ± 0.4	0 ± 0	0 ± 0	0 ± 0	0.25 ± 0.79	0 ± 0
6	0.01 ± 0.04	0 ± 0	0 ± 0	0.03 ± 0.08	0.01 ± 0.04	0 ± 0	0 ± 0	0.06 ± 0.2
7	0.03 ± 0.08	0 ± 0	0 ± 0	0.14 ± 0.36	0.05 ± 0.16	0 ± 0	0 ± 0	0.34 ± 0.87
8	0 ± 0	0.03 ± 0.08	0 ± 0	0.01 ± 0.04	0 ± 0	0.03 ± 0.08	0 ± 0	0.01 ± 0.04
9	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
10	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

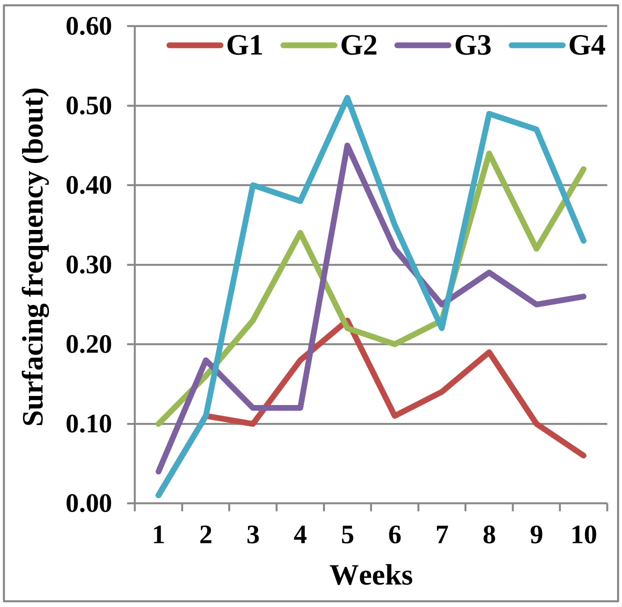

Fig. (1): Interaction plot of surfacing frequency (bout) for the four experimental groups during ten weeks of experiment.

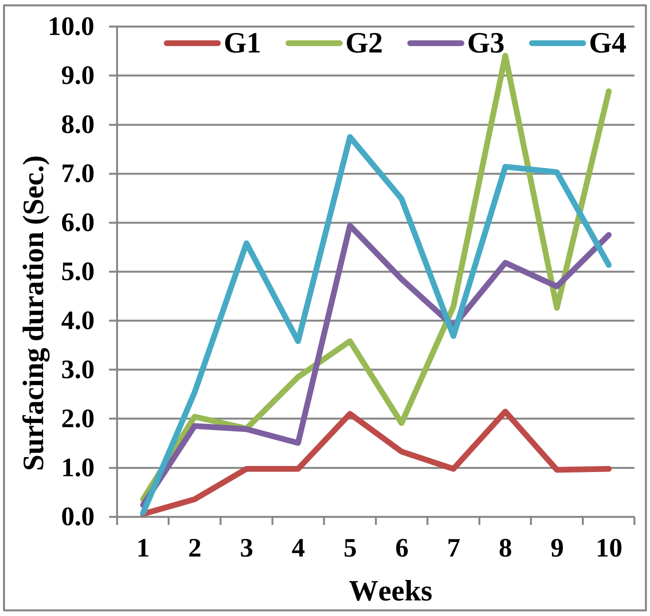

Fig. (2): Interaction plot of surfacing duration (sec.) for the four experimental groups during ten weeks of experiment.

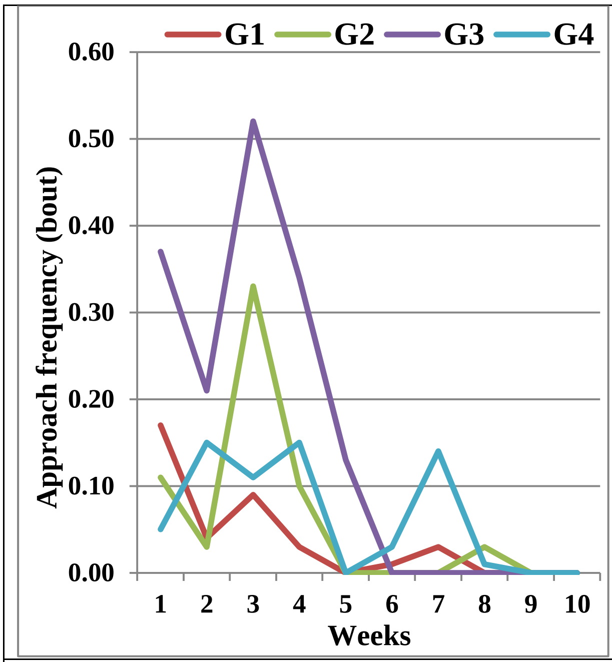

Fig. (3): Interaction plot of approach frequency (bout) for the four experimental groups during ten weeks of experiment.

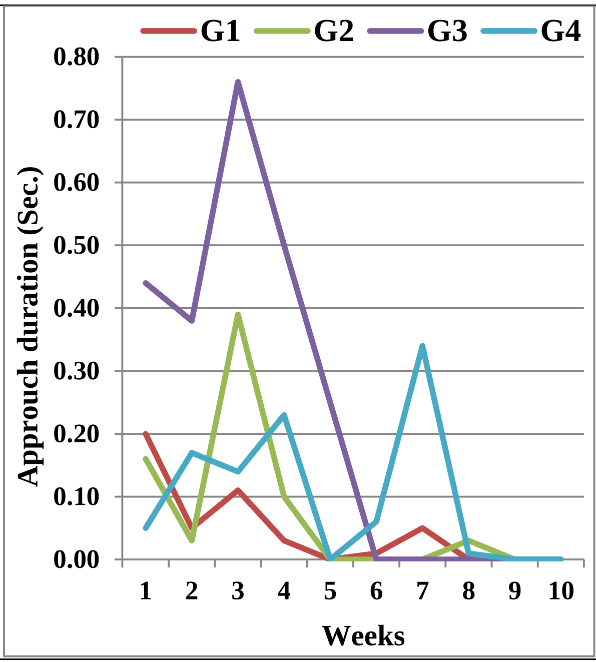

Fig. (4): Interaction plot of Approach duration (sec.) for the four experimental groups during ten weeks of experiment.

Chasing behavior (Frequency and duration):

Results **(Table 5)** showed that the effect of weeks of experiment produced a significant difference in reading of chasing frequency among groups (P< **0.05**). By looking to interaction plot **(Fig. 5)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest chasing frequency behavior recorded was in G_3 (**1.3±0.52 bout**), and its lowest value was recorded in G_4. By going on weeks of experiments it showed a decrease during week 5 in G_1, but G_4 was the highest. In the last week, G_1 was the only group showed an increase in this behavior, but G_4 was still the highest. Generally, interaction plot showed that G_1 showed the minimum value **(0.06 ± 0.11 bout)** of chasing behavior compared to the other three groups, and G_3 showed the maximum value **(1.98 ± 1.54 bout)** after the first week of experiment.

Results **(Table 5)** showed that the effect of weeks of experiment produced a significant difference in reading of chasing duration among groups (P< **0.01**). By looking to interaction plot **(Fig. 6)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest chasing duration behavior recorded was in G_3 (**1.71±0.73** sec.), and its lowest value was recorded in G_4. By going on weeks of experiments it showed a decrease during week 5 in G_1, but G_4 was the highest. In the last week, G_1 showed an increase in this behavior but G_4 was still the highest. Generally, interaction plot showed that G_1 showed the minimum value **(0.09 ± 0.15 sec.)** of chasing behavior compared to the other three groups, and G_3 showed the maximum value **(4.05 ± 1.86 sec.)** after the first week of experiment.

Fin Tugging behavior (Frequency and duration):

Results showed that the effect of weeks of experiment produced insignificant difference in reading of fin tugging frequency among groups P- value (**0.8**) By looking to interaction plot **(Fig.7)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest fin tugging behavior recorded was in the group3 (**0.09±0.1**), the behavior in group 2 and 4 was lower in average than group3, and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior showed in week 5 an increase only in group 3 and showed a decrease in other groups. In the last week group 2 was the only group showed an increase in the behavior while the other groups showed a decrease. The general look to interaction plot showed that group **1(0.01 ± 0.04)** showed the minimum values of fin tugging behavior compared to the other three groups, and group **4 (0.09 ± 0.16)** nearly showed the highest values after the first week of experiment **(Table 6)**.

Results showed that the effect of weeks of experiment produced insignificant difference in reading of fin tugging duration among groups P- value (**0.6**) By looking to interaction plot **(Fig. 8)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest fin tugging behavior recorded was in the group3 (**0.11±0.14**), the behavior in group 2 and 4 was lower in average than group3, and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior showed a decrease in week 4 in group1, 2, and 3 and showed an increase in group 4. In the last week group 2 was the only group showed an increase in the behavior while the other three groups showed a decrease .The general look to interaction plot showed that group **1 (0.01 ± 0.04)** showed the minimum values of fin tugging behavior compared to the other three groups, and group **3 (0.25 ± 0.41)**, **4 (0.15 ± 0.47**) nearly showed the highest values after the first week of experiment **(Table 6)**.

Table (5): Mean ± SD of Chasing behavior in the four groups during ten weeks of experiment.

Weeks	Chasing Frequency				Chasing Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.56 ± 0.65	0.32 ± 0.3	1.3 ± 0.52	0.29 ± 0.62	1.12 ± 1.45	0.44 ± 0.47	1.71 ± 0.73	0.4 ± 0.93
2	0.45 ± 0.42	0.65 ± 0.72	1.28 ± 0.68	0.74 ± 0.51	0.78 ± 0.61	1.14 ± 1.38	2.53 ± 1.26	1.29 ± 0.93
3	0.27 ± 0.27	0.83 ± 0.98	1.98 ± 1.54	1.54 ± 1.37	0.45 ± 0.47	1.37 ± 1.55	3.73 ± 2.85	3.18 ± 2.92
4	0.09 ± 0.19	0.28 ± 0.42	1.75 ± 1.62	0.96 ± 1.77	0.13 ± 0.27	0.41 ± 0.67	3.33 ± 3.05	2.09 ± 4.14
5	0.18 ± 0.33	0.58 ± 0.79	1.15 ± 0.91	1.38 ± 1.09	0.23 ± 0.44	1.31 ± 1.72	2.83 ± 2.25	3.54 ± 2.86
6	0.15 ± 0.27	0.49 ± 0.4	1.62 ± 0.81	1.05 ± 0.83	0.21 ± 0.4	1.05 ± 0.85	4.05 ± 1.86	1.88 ± 1.75
7	0.06 ± 0.11	0.28 ± 0.34	0.35 ± 0.5	0.6 ± 0.39	0.09 ± 0.15	0.58 ± 0.73	0.94 ± 1.49	1.46 ± 1.05
8	0.13 ± 0.21	0.15 ± 0.25	1.33 ± 0.96	0.44 ± 0.66	0.19 ± 0.32	0.4 ± 0.69	3.33 ± 2.53	0.96 ± 1.54
9	0.24 ± 0.25	0.35 ± 0.39	0.74 ± 0.75	0.67 ± 1.53	0.34 ± 0.29	0.74 ± 1	2.22 ± 2.43	1.65 ± 4.05
10	0.21 ± 0.43	0.27 ± 0.25	0.58 ± 0.59	0.6 ± 0.77	0.55 ± 1.32	0.57 ± 0.56	1.9 ± 2.13	2.16 ± 2.94

Table (6): Mean ± SD of Fin Tugging behavior in the four groups during ten weeks of experiment.

Weeks	Fin Tugging Frequency				Fin Tugging Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.03 ± 0.05	0.05 ± 0.07	0.09 ± 0.1	0.05 ± 0.09	0.04 ± 0.08	0.06 ± 0.09	0.11 ± 0.14	0.05 ± 0.09
2	0.01 ± 0.04	0.04 ± 0.08	0.1 ± 0.14	0.06 ± 0.11	0.03 ± 0.08	0.05 ± 0.12	0.25 ± 0.41	0.1 ± 0.2
3	0 ± 0	0.03 ± 0.05	0.05 ± 0.09	0.09 ± 0.16	0 ± 0	0.03 ± 0.05	0.06 ± 0.12	0.09 ± 0.16
4	0 ± 0	0 ± 0	0.08 ± 0.09	0.09 ± 0.16	0 ± 0	0 ± 0	0.08 ± 0.09	0.09 ± 0.16
5	0 ± 0	0 ± 0	0.09 ± 0.17	0.04 ± 0.06	0 ± 0	0 ± 0	0.13 ± 0.22	0.04 ± 0.06
6	0 ± 0	0.01 ± 0.04	0.04 ± 0.08	0.04 ± 0.08	0 ± 0	0.03 ± 0.08	0.04 ± 0.08	0.1 ± 0.28
7	0.01 ± 0.04	0 ± 0	0.06 ± 0.14	0.08 ± 0.17	0.01 ± 0.04	0 ± 0	0.11 ± 0.25	0.13 ± 0.27
8	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
9	0 ± 0	0 ± 0	0.03 ± 0.08	0.08 ± 0.24	0 ± 0	0 ± 0	0.05 ± 0.16	0.15 ± 0.47
10	0.03 ± 0.05	0.05 ± 0.07	0.09 ± 0.1	0.05 ± 0.09	0.04 ± 0.08	0.06 ± 0.09	0.11 ± 0.14	0.05 ± 0.09

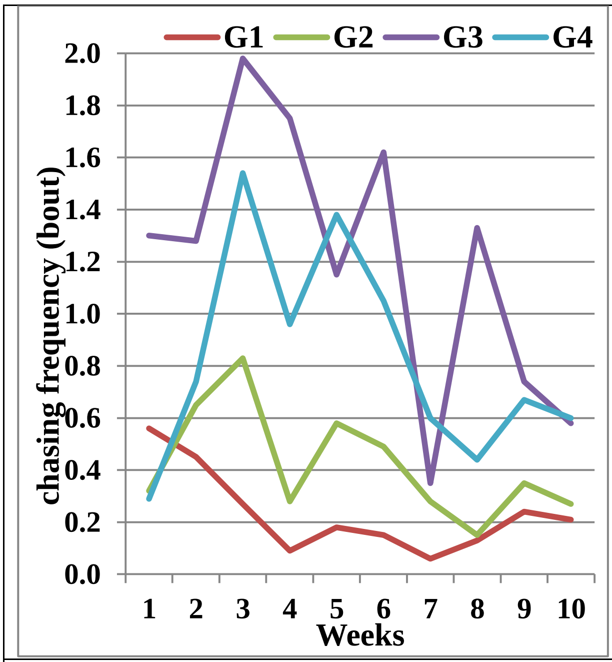

Fig. (5): Interaction plot of Chasing frequency for four groups during ten weeks of experiment.

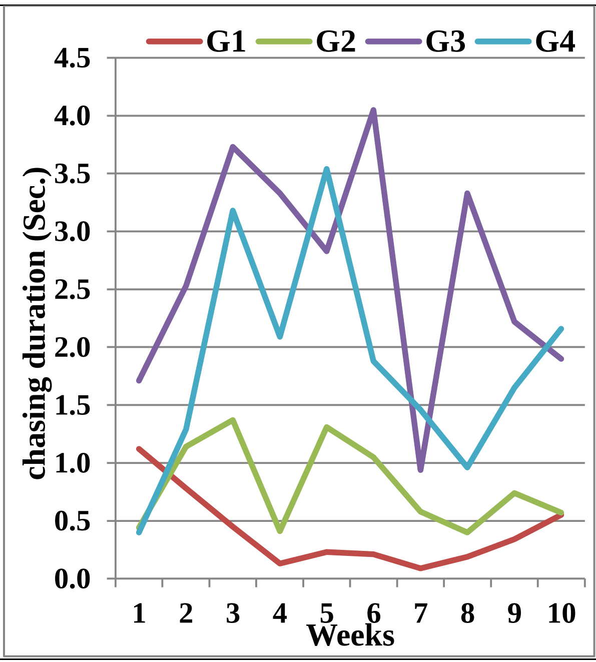

Fig. (6): Interaction plot of Chasing duration for four groups during ten weeks of experiment.

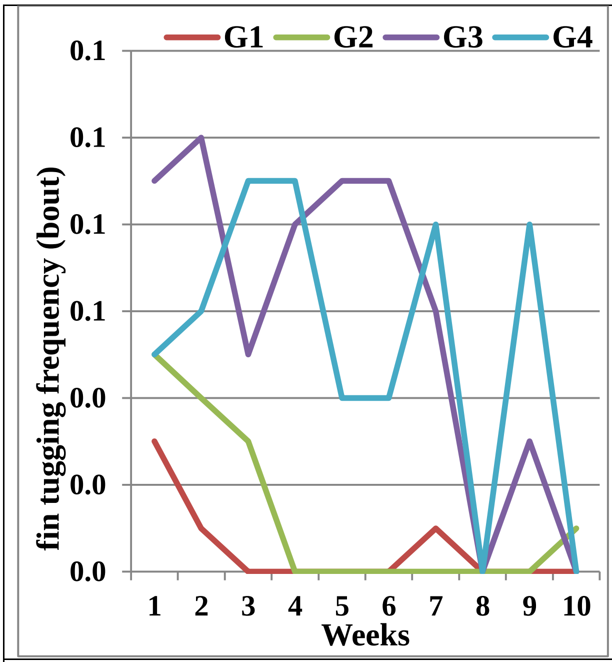

Fig. (7): Interaction plot of Fin Tugging frequency for four groups during ten weeks of experiment.

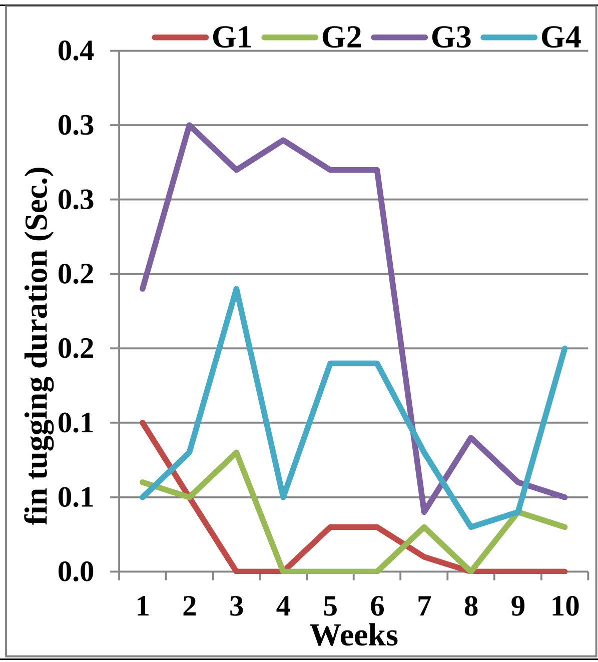

Fig. (8): Interaction plot of Fin Tugging duration for four groups during ten weeks of experiment.

Biting behavior (Frequency and duration)

Results **(Table 7)** showed that the effect of weeks of experiment significantly produced a significant difference in reading of biting frequency among groups P- value (**0.03**) By looking to interaction plot **(Fig. 9)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest biting behavior recorded was in the group3 **(0.22±0.22)**, the behavior in group 1, 4 was lower in average than group 3 and the lowest behavior was recorded in group 2. By going on weeks of experiments the behavior showed a decrease in week 5 in group 1 and showed an increase in other groups but group 3 was still the highest. In the last week group1, 4 showed an increase in the behavior while the other groups showed a decrease. The general look to interaction plot showed that group **1 (0.01 ± 0.04)** showed the minimum values of biting behavior compared to the other three groups, and group **3 (0.45 ± 0.27**) nearly showed the highest values after the first week of experiment**.**

Results **(Table 7)** showed that the effect of weeks of experiment significantly produced a significant difference in reading of biting duration among groups P- value (**0.005**) By looking to interaction plot **(Fig. 10)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest biting behavior recorded was in the group3 **(0.25±0.26),** the behavior in group 1, 4 was lower in average than group3, and the lowest behavior was recorded in group 2. By going on weeks of experiments the behavior showed a decrease in week 5 in group 1 and showed an increase in other groups but group 3 was still the highest. In the last week group 4 showed more increase in the behavior than group 1 while the other groups showed a decrease. The general look to interaction plot showed that group **1 (0.01 ± 0.04)** showed the minimum values of biting behavior compared to the other three groups, and group **3 (0.69 ± 0.39)** nearly showed the highest values after the first week of experiment.

Butting behavior (Frequency and duration)

Results showed that the effect of weeks of experiment produced insignificant difference in reading of butting frequency among groups P- value (**0.1**) By looking to interaction plot **(Fig. 11)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest butting behavior recorded was in the group3 (**0.3±0.23**), the behavior in group 2, 4 was lower in average than group3 and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior in week 5 showed an increase in group2 while showed a decrease in other groups but group 3 was still the highest. In the last week the behavior showed a decrease in all groups but group 4 was the highest. The general look to interaction plot showed that group **1 (0.01 ± 0.04)** showed the minimum values of butting behavior compared to the other three groups, and group **3 (0.3 ± 0.23)** nearly showed the highest values after the first week of experiment **(Table 8)**.

Results showed that the effect of weeks of experiment produced insignificant difference in reading of butting frequency among groups P- value (**0.08**) By looking to interaction plot **(Fig. 12)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest butting behavior recorded was in the group3 **(0.43±0.34**), the behavior in group 2, 4 was lower in average than group 3 and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior in week 5 showed an increase in group2 while showed a decrease in other groups but group 3 was still the highest. In the last week the behavior showed a decrease in all groups but group 4 was the highest. The general look to interaction plot showed that group **1(0.01 ± 0.04)** showed the minimum values of butting behavior compared to the other three groups, and group **3 (0.43 ± 0.34)** nearly showed the highest values after the first week of experiment **(Table 8)**.

Table (7): Mean ± SD of Biting behavior in the four groups during ten weeks of experiment.

Weeks	Biting Frequency				Biting Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.06 ± 0.12	0.05 ± 0.07	0.22 ± 0.22	0.06 ± 0.12	0.09 ± 0.2	0.05 ± 0.07	0.25 ± 0.26	0.06 ± 0.12
2	0.08 ± 0.16	0.13 ± 0.13	0.27 ± 0.22	0.14 ± 0.12	0.08 ± 0.16	0.13 ± 0.13	0.35 ± 0.38	0.14 ± 0.12
3	0.03 ± 0.05	0.3 ± 0.3	0.34 ± 0.21	0.24 ± 0.27	0.04 ± 0.08	0.36 ± 0.4	0.44 ± 0.35	0.3 ± 0.35
4	0.03 ± 0.08	0.06 ± 0.12	0.34 ± 0.39	0.24 ± 0.52	0.05 ± 0.16	0.06 ± 0.12	0.37 ± 0.42	0.25 ± 0.53
5	0 ± 0	0.18 ± 0.26	0.45 ± 0.27	0.2 ± 0.24	0 ± 0	0.26 ± 0.37	0.69 ± 0.39	0.28 ± 0.31
6	0.03 ± 0.08	0.09 ± 0.1	0.38 ± 0.28	0.24 ± 0.2	0.05 ± 0.16	0.2 ± 0.27	0.56 ± 0.46	0.26 ± 0.21
7	0.03 ± 0.05	0.03 ± 0.08	0.1 ± 0.16	0.14 ± 0.15	0.04 ± 0.08	0.03 ± 0.08	0.15 ± 0.24	0.21 ± 0.24
8	0.03 ± 0.05	0.05 ± 0.11	0.23 ± 0.18	0.03 ± 0.08	0.04 ± 0.08	0.06 ± 0.14	0.3 ± 0.24	0.03 ± 0.08
9	0.01 ± 0.04	0.06 ± 0.11	0.16 ± 0.19	0.08 ± 0.24	0.01 ± 0.04	0.1 ± 0.17	0.31 ± 0.37	0.1 ± 0.32
10	0.01 ± 0.04	0.06 ± 0.11	0 ± 0	0.2 ± 0.27	0.01 ± 0.04	0.09 ± 0.15	0 ± 0	0.38 ± 0.46

Table (8): Mean ± SD of Butting behavior in the four groups during ten weeks of experiment.

Weeks	Butting Frequency				Butting Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.04 ± 0.06	0.09 ± 0.1	0.3 ± 0.23	0.14 ± 0.18	0.06 ± 0.1	0.13 ± 0.2	0.43 ± 0.34	0.16 ± 0.22
2	0.01 ± 0.04	0.13 ± 0.24	0.19 ± 0.16	0.13 ± 0.12	0.01 ± 0.04	0.2 ± 0.43	0.19 ± 0.16	0.13 ± 0.12
3	0 ± 0	0.11 ± 0.16	0.21 ± 0.21	0.2 ± 0.25	0 ± 0	0.11 ± 0.16	0.25 ± 0.28	0.26 ± 0.36
4	0 ± 0	0.01 ± 0.04	0.29 ± 0.32	0.09 ± 0.21	0 ± 0	0.01 ± 0.04	0.4 ± 0.43	0.09 ± 0.21
5	0 ± 0	0.09 ± 0.13	0.23 ± 0.3	0.09 ± 0.12	0 ± 0	0.14 ± 0.19	0.35 ± 0.49	0.09 ± 0.12
6	0.01 ± 0.04	0.05 ± 0.11	0.21 ± 0.26	0.16 ± 0.17	0.03 ± 0.08	0.08 ± 0.17	0.36 ± 0.44	0.16 ± 0.2
7	0 ± 0	0 ± 0	0.05 ± 0.11	0.15 ± 0.28	0 ± 0	0 ± 0	0.06 ± 0.14	0.26 ± 0.48
8	0 ± 0	0.04 ± 0.08	0.14 ± 0.12	0 ± 0	0 ± 0	0.06 ± 0.14	0.19 ± 0.18	0 ± 0
9	0 ± 0	0.02 ± 0.08	0.08 ± 0.12	0.04 ± 0.12	0 ± 0	0.03 ± 0.08	0.15 ± 0.24	0.06 ± 0.2
10	0 ± 0	0.03 ± 0.08	0 ± 0	0.04 ± 0.08	0 ± 0	0.05 ± 0.16	0 ± 0	0.06 ± 0.16

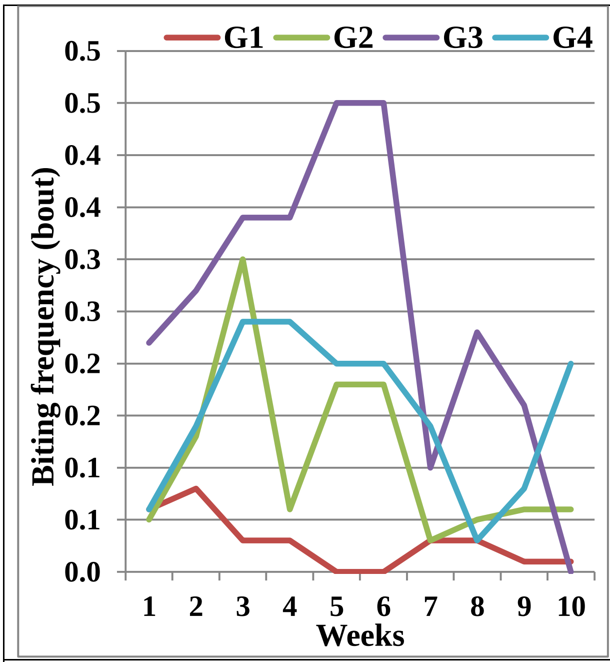

Fig. (9): Interaction plot of Biting frequency for four groups during ten weeks of experiment.

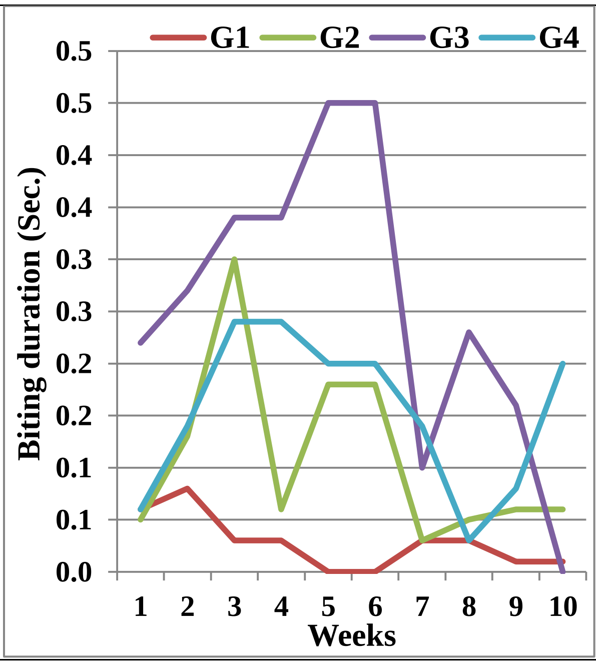

Fig. (10): Interaction plot of Biting duration for four groups during ten weeks of experiment.

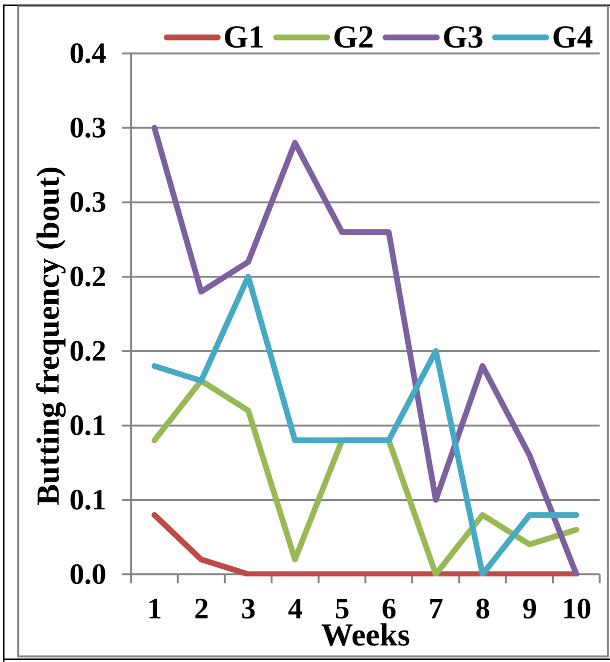

Fig. (11): Interaction plot of Butting frequency for four groups during ten weeks of experiment.

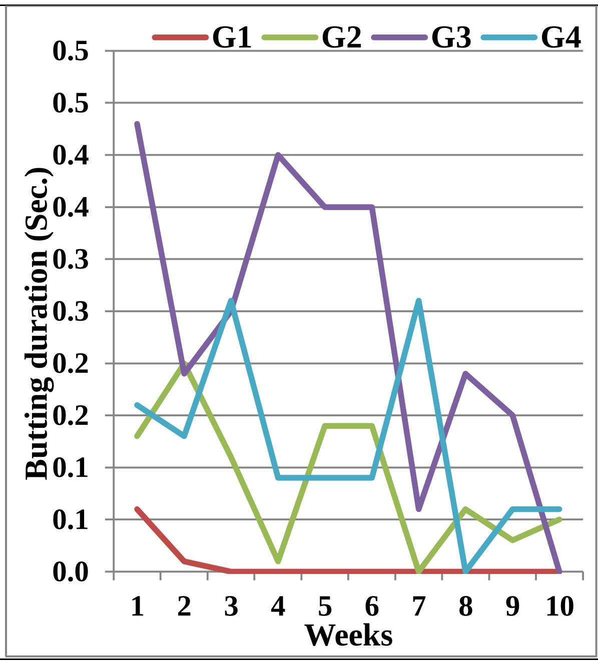

Fig. (12): Interaction plot of Butting duration for four groups during ten weeks of experiment.

Fleeing behavior (Frequency and duration)

Results **(Table 9)** showed that the effect of weeks of experiment produced significantly produced a significant difference in reading of fleeing frequency among groups P- value (**0.001**) By looking to interaction plot **(Fig. 13)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest fleeing behavior recorded was in the group3 **(1.03 ± 0.39),** the behavior in group 1, 4 was lower in average than group3, and the lowest behavior was recorded in group 2. By going on weeks of experiments the behavior showed a decrease in week 5 in all groups but group 4 was the highest. In the last week the behavior showed more decrease in all groups but group 4 was still the highest. The general look to interaction plot showed that group **1 (0.11 ± 0.2)** showed the minimum values of fleeing behavior compared to the other three groups, and group **3 (1.17 ± 0.49)** nearly showed the highest values after the first week of experiment.

Results in **Table (9)** shows that the effect of weeks of experiment produced a significant difference in reading of fleeing duration among groups P- value (**0.001**). By looking to interaction plot **(Fig. 14)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest fleeing behavior recorded was in the group 4 **(1.21 ± 0.95**), the behavior in groups 1and 3 was lower in average than group4, and the lowest behavior was recorded in group 2. By going on weeks of experiments the behavior showed a decrease in week 5 in group 1, 3 while showed an increase in groups 2and 4, but group 4 was the highest. In the last week the behavior showed more decrease in all groups but group 4 was still the highest. The general look to interaction plot showed that group **1 (1.04 ± 0.58)** showed the minimum values of fleeing behavior compared to the other three groups, and group **3 (2.44 ± 1.08)** and **4 (2.59 ± 1.59)** nearly showed the highest values after the first week of experiment.

Mouth pushing behavior (Frequency and duration)

Results **(Table 10)** showed that the effect of weeks of experiment significantly produced a significant difference in reading of mouth pushing frequency among groups P- value (**0.002**) By looking to interaction plot **(Fig. 15)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest mouth pushing recorded was in the group3 (**0.19±0.18**), the behavior in group 2, 4 was lower in average than group3, and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior in week 5 showed a decrease in group 1, 2 and showed an increase in group3, 4 but group 3 was still the highest. In the last week group 4 showed more increase in the behavior than group 2 while the other groups showed a decrease. The general look to interaction plot showed that group **1(0.01 ± 0.04)** showed the minimum values of mouth pushing behavior compared to the other three groups, and group **3 (0.3 ± 0.26)** nearly showed the highest values after the first week of experiment.

Results **(Table 10)** showed that the effect of weeks of experiment significantly produced a significant difference in reading of mouth pushing duration among groups P- value (**0.001**) By looking to interaction plot **(Fig. 16)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest mouth pushing recorded was in the group3 (**0.49±0.56**), the behavior in group 2, 4 was lower in average than group3, and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior showed in week 6 a marked increase in group 4 than group 3 and showed a decrease in other groups. In the last week group 2 was the only group showed an increase in the behavior while the other 3 groups showed a decrease but group 4 was still the highest. The general look to interaction plot showed that group **1 (0.01 ± 0.04)** showed the minimum values of mouth pushing behavior compared to the other three groups, and group **3 (0.8 ± 0.67)** nearly showed the highest values after the first week of experiment.

Table (9): Mean ± SD of Fleeing behavior in the four groups during ten weeks of experiment.

Weeks	Fleeing Frequency				Fleeing Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.78 ± 0.32	0.6 ± 0.46	1.03 ± 0.39	0.94 ± 0.73	1.17 ± 0.54	0.93 ± 0.87	1.21 ± 0.52	1.21 ± 0.95
2	0.841 ± 0.38	0.29 ± 0.31	1.17 ± 0.49	0.84 ± 0.3	1.83 ± 1.12	0.42 ± 0.46	2.44 ± 1.08	1.68 ± 0.49
3	0.67 ± 0.39	0.52 ± 0.38	0.98 ± 0.47	1.29 ± 0.74	1.04 ± 0.58	1.03 ± 0.81	1.97 ± 1.04	2.59 ± 1.59
4	0.23 ± 0.24	0.35 ± 0.14	1.14 ± 0.62	0.37 ± 0.28	0.3 ± 0.34	0.71 ± 0.27	2.14 ± 0.88	0.64 ± 0.48
5	0.24 ± 0.21	0.45 ± 0.31	0.53 ± 0.39	0.83 ± 0.38	0.33 ± 0.24	1.03 ± 0.88	1.12 ± 0.85	1.77 ± 0.98
6	0.11 ± 0.2	0.19 ± 0.19	0.56 ± 0.37	0.44 ± 0.44	0.21 ± 0.4	0.35 ± 0.35	1.23 ± 0.94	0.89 ± 1.08
7	0.19 ± 0.11	0.2 ± 0.19	0.45 ± 0.33	0.34 ± 0.29	0.32 ± 0.24	0.4 ± 0.31	1.04 ± 0.7	0.66 ± 0.52
8	0.23 ± 0.19	0.15 ± 0.14	0.68 ± 0.32	0.45 ± 0.3	0.34 ± 0.27	0.36 ± 0.33	1.4 ± 0.57	0.94 ± 0.61
9	0.24 ± 0.13	0.18 ± 0.19	0.34 ± 0.3	0.4 ± 0.32	0.32 ± 0.16	0.33 ± 0.37	0.9 ± 0.87	0.92 ± 0.8
10	0.18 ± 0.16	0.21 ± 0.23	0.35 ± 0.18	0.48 ± 0.18	0.28 ± 0.24	0.51 ± 0.63	0.81 ± 0.49	1.16 ± 0.5

Table (10): Mean ± SD of Mouth Pushing behavior in the four groups during ten weeks of experiment.

Weeks	Mouth Pushing Frequency				Mouth Pushing Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.1 ± 0.1	0.06 ± 0.09	0.19 ± 0.18	0.05 ± 0.09	0.21 ± 0.23	0.11 ± 0.2	0.49 ± 0.56	0.09 ± 0.17
2	0.05 ± 0.09	0.05 ± 0.09	0.3 ± 0.26	0.08 ± 0.11	0.08 ± 0.14	0.1 ± 0.2	0.7 ± 0.6	0.15 ± 0.21
3	0 ± 0	0.08 ± 0.17	0.27 ± 0.19	0.19 ± 0.15	0 ± 0	0.1 ± 0.22	0.8 ± 0.67	0.39 ± 0.32
4	0 ± 0	0 ± 0	0.29 ± 0.19	0.05 ± 0.09	0 ± 0	0 ± 0	0.69 ± 0.45	0.09 ± 0.17
5	0.03 ± 0.05	0 ± 0	0.27 ± 0.33	0.14 ± 0.27	0.04 ± 0.08	0 ± 0	0.76 ± 1.08	0.35 ± 0.73
6	0.01 ± 0.04	0.03 ± 0.05	0.19 ± 0.25	0.24 ± 0.22	0.01 ± 0.04	0.05 ± 0.11	0.58 ± 0.88	1.13 ± 1.17
7	0.01 ± 0.04	0.03 ± 0.05	0.04 ± 0.08	0.08 ± 0.11	0.01 ± 0.04	0.05 ± 0.11	0.08 ± 0.17	0.18 ± 0.25
8	0 ± 0	0 ± 0	0.09 ± 0.17	0.03 ± 0.05	0 ± 0	0 ± 0	0.34 ± 0.72	0.06 ± 0.14
9	0 ± 0	0.04 ± 0.08	0.06 ± 0.09	0.04 ± 0.08	0 ± 0	0.09 ± 0.21	0.23 ± 0.32	0.1 ± 0.21
10	0 ± 0	0.03 ± 0.05	0.05 ± 0.11	0.15 ± 0.19	0 ± 0	0.06 ± 0.14	0.13 ± 0.27	0.44 ± 0.52

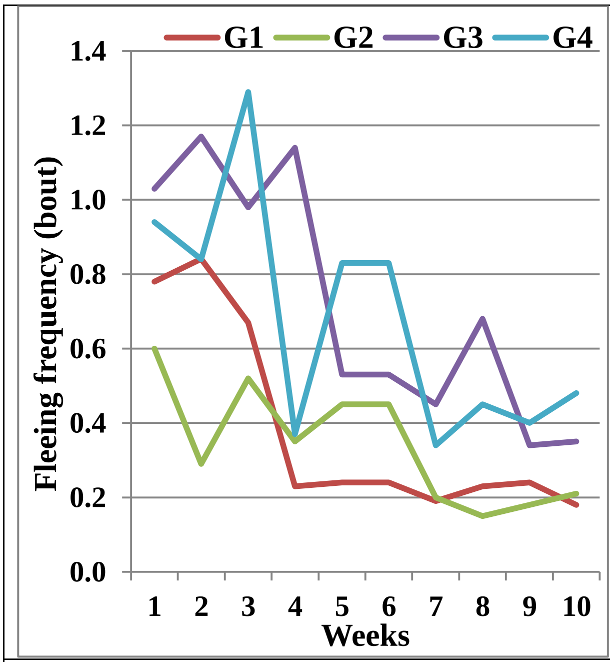

Fig. (13): Interaction plot of fleeing frequency for four groups during ten weeks of experiment.

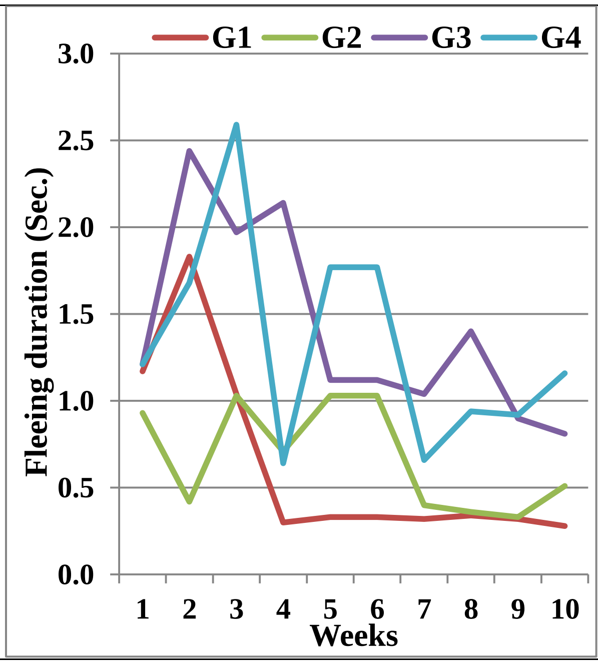

Fig. (14): Interaction plot of fleeing duration for four groups during ten weeks of experiment.

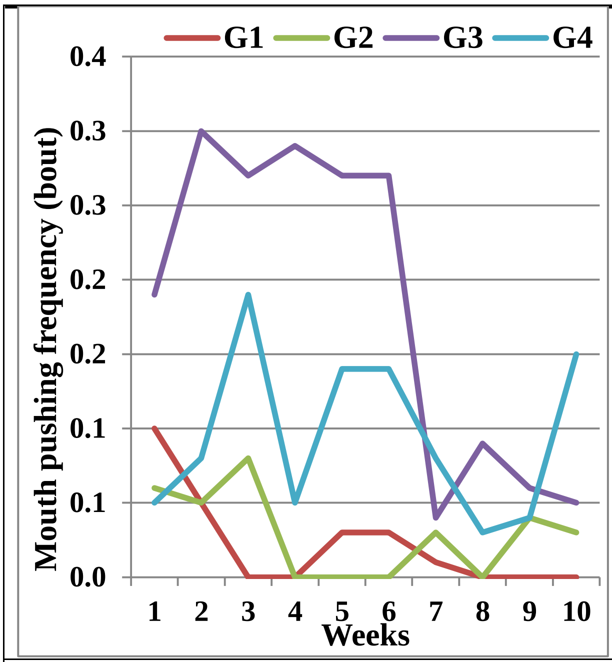

Fig. (15): Interaction plot of Mouth Pushing frequency for four groups during ten weeks of experiment.

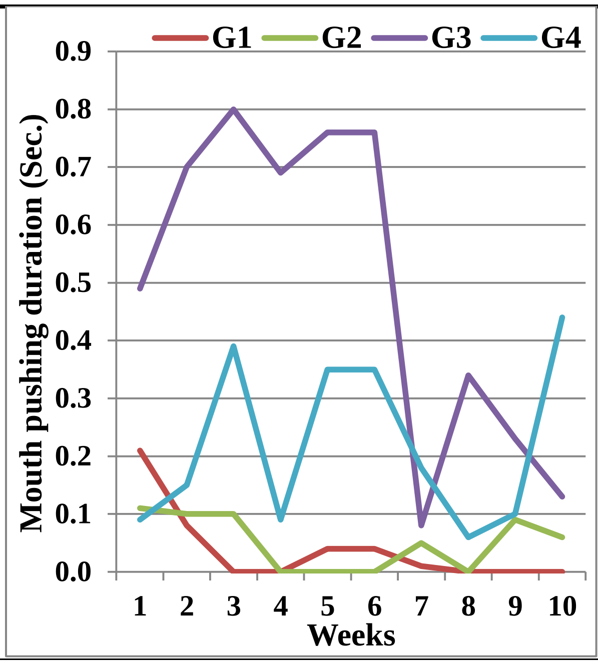

Fig. (16): Interaction plot of Mouth Pushing duration for four groups during ten weeks of experiment.

Spreading of Fins behavior (Frequency and duration)

Results **(Table 11)** showed that the effect of weeks of experiment significantly produced a significant difference in reading of spreading of fins frequency among groups P- value (**0.001**) By looking to interaction plot **(Fig. 17)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest spreading of fins recorded was in the group3 **(0.15±0.43),** the behavior in group 2, 4 was lower in average than group 3 and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior showed a decrease in week 5 in groups 1, 2 and showed more increase in group 4 than group 3. In the last week all groups showed a decrease in the behavior but group 4 was still the highest. The general look to interaction plot showed that group **1 (0.01 ± 0.04)** showed the minimum values of spreading of fins behavior compared to the other three groups, and group **3 (0.83 ± 0.72)** nearly showed the highest values after the first week of experiment.

Results **(Table 11)** showed that the effect of weeks of experiment significantly produced a significant difference in reading of spreading of fins duration among groups P- value (**0.001**) By looking to interaction plot **(Fig. 18)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest spreading of fins recorded was in the group3 **(0.45±0.32),** the behavior in group 2,4 was lower in average than group3, and the lowest behavior was recorded in group 1. By going on weeks of experiments the behavior showed a decrease in week 5 in groups 1, 2 and showed more increase in group 4 than group 3. In the last week all groups showed a decrease in the behavior but group 4 was still the highest. The general look to interaction plot showed that group **1 (0.01 ± 0.04)** showed the minimum values of spreading of fins behavior compared to the other three groups, and group **3 (4.14 ± 3.36)** nearly showed the highest values after the first week of experiment.

Table (11): Mean ± SD of Spreading of Fins behavior in the four groups during ten weeks of experiment.

Weeks	Spreading of Fins Frequency				Spreading of Fins Duration			
	G1	G2	G3	G4	G1	G2	G3	G4
1	0.03 ± 0.05	0.08 ± 0.14	0.23 ± 0.13	0.15 ± 0.43	0.05 ± 0.12	0.31 ± 0.63	0.45 ± 0.32	0.18 ± 0.51
2	0.09 ± 0.12	0.14 ± 0.22	0.4 ± 0.35	0.14 ± 0.16	0.43 ± 0.79	0.49 ± 0.73	1.52 ± 1.29	0.38 ± 0.44
3	0 ± 0	0.08 ± 0.17	0.43 ± 0.33	0.31 ± 0.38	0 ± 0	0.4 ± 0.88	1.87 ± 1.58	1.38 ± 1.68
4	0.01 ± 0.04	0 ± 0	0.83 ± 0.72	0.24 ± 0.39	0.06 ± 0.2	0 ± 0	4.14 ± 3.36	1.29 ± 2.12
5	0 ± 0	0.01 ± 0.04	0.42 ± 0.38	0.54 ± 0.49	0 ± 0	0.13 ± 0.4	2.28 ± 1.97	2.78 ± 2.8
6	0.01 ± 0.04	0 ± 0	0.3 ± 0.28	0.25 ± 0.27	0.01 ± 0.04	0 ± 0	1.93 ± 1.93	1.81 ± 1.74
7	0 ± 0	0 ± 0	0.06 ± 0.11	0.08 ± 0.11	0 ± 0	0 ± 0	0.58 ± 1.05	0.6 ± 0.88
8	0 ± 0	0.01 ± 0.04	0.19 ± 0.24	0.1 ± 0.14	0 ± 0	0.25 ± 0.79	1.46 ± 1.51	1.35 ± 2.32
9	0 ± 0	0.01 ± 0.04	0.08 ± 0.11	0.15 ± 0.39	0 ± 0	0.09 ± 0.28	0.64 ± 0.91	0.63 ± 1.77
10	0 ± 0	0.01 ± 0.04	0.08 ± 0.09	0.24 ± 0.29	0 ± 0	0.1 ± 0.32	0.88 ± 1.24	1.55 ± 1.75

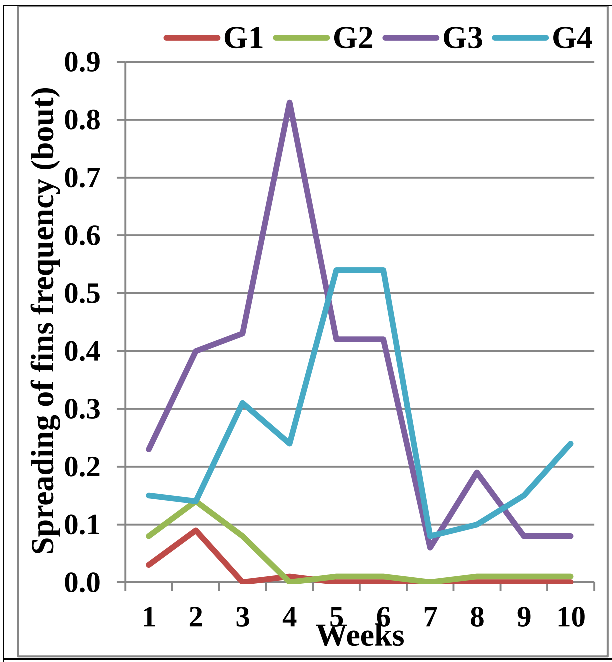

Fig. (17): Interaction plot of Spreading of Fins frequency for four groups during ten weeks of experiment.

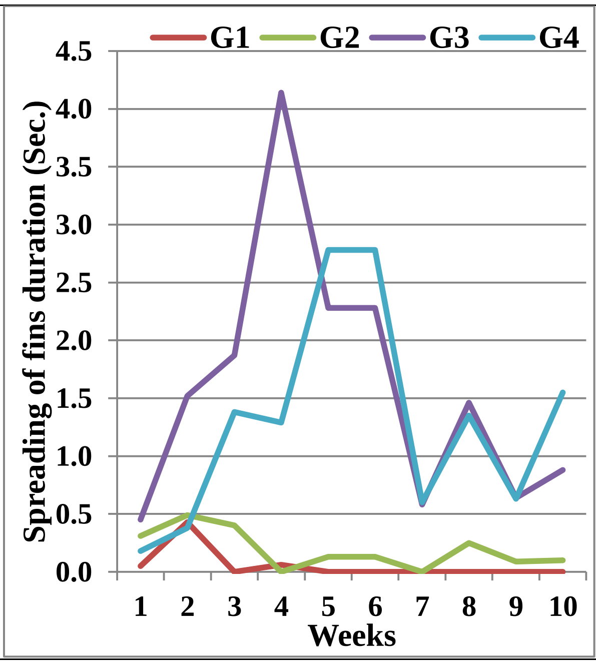

Fig. (18): Interaction plot of Spreading of Fins duration for four groups during ten weeks of experiment.

<u>Crossing test</u>

Results **(Table 12)** showed that the effect of weeks of experiment significantly produced significant differences in reading of crossing test among groups P- value (**0.001**). By looking to interaction plot **(Fig. 19)** that clearly showed the differences among groups during the weeks of experiment, in the first week the highest crossing test value recorded was in the group2 (**2.93±1.26**), the behavior in group1,4 was lower in average than group2 and the lowest behavior was recorded in group 3. By going on weeks of experiments the crossing test showed an increase in group1, while showed a decrease in other groups. In the last week, group 1 showed a decrease but was still the highest in the crossing test while the other 3 groups showed an increase. The general look to interaction plot showed that group **3 (0.65 ± 0.23**) showed the minimum values of crossing test compared to the other three groups, and group **1 (3.18 ± 0.40)** nearly showed the highest values after the first week of experiment.

Table (12): Mean ± SD of crossing test in the four groups during ten weeks of experiment.

Weeks	G1	G2	G3	G4
1	2.00 ± 0.83	2.93 ± 1.26	0.83 ± 0.22	1.35 ± 0.34
2	2.91 ± 0.52	1.91 ± 0.66	0.98 ± 0.28	0.89 ± 0.10
3	3.18 ± 0.40	1.92 ± 0.23	0.91 ± 0.15	0.93 ± 0.08
4	1.46 ± 0.35	1.45 ± 0.25	0.77 ± 0.15	0.74 ± 0.19
5	2.61 ± 0.90	1.41 ± 0.38	0.68 ± 0.08	0.68 ± 0.19
6	1.63 ± 0.48	1.73 ± 0.36	0.82 ± 0.15	0.82 ± 0.11
7	1.84 ± 0.52	1.25 ± 0.19	0.65 ± 0.23	0.63 ± 0.12
8	2.83 ± 0.77	1.60 ± 0.33	0.68 ± 0.14	0.75 ± 0.13
9	2.19 ± 0.58	1.34 ± 0.32	0.71 ± 0.12	0.75 ± 0.13
10	2.21 ± 0.72	1.82 ± 0.25	0.81 ± 0.16	0.85 ± 0.13

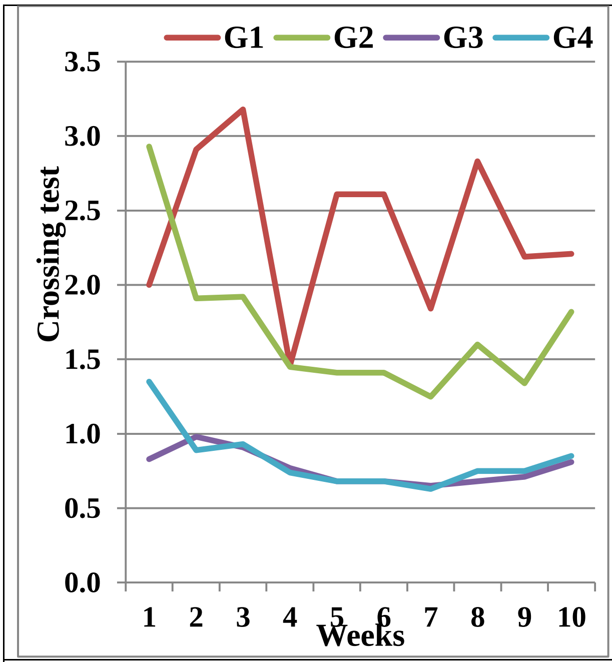

Fig. (19): Interaction plot of crossing test for four groups during ten weeks of experiment.

Table (13): Mean ± SD of Effect of stocking density on average body weight per gram.

Weeks	G1	G2	G3	G4	Sig.
Initial w	31.28 ± 0.82	31.33 ± 0.78	31.38 ± 0.75	31.46 ± 0.75	0.999
2w	36.66 ± 0.54[a]	35.19 ± 0.33[ab]	35.81 ± 0.29[ab]	35.03 ± 0.39[b]	0.039
4w	39.91 ± 0.49[a]	39.12 ± 0.17[a]	36.65 ± 0.38[b]	35.31 ± 0.34[c]	0.001
6w	41.47 ± 1.23[ab]	43.02 ± 0.96[a]	39.53 ± 0.93[c]	37.24 ± 0.97[c]	0.001
8w	44.84 ± 1.42[a]	43.20 ± 0.98[ab]	40.52 ± 0.87[bc]	38.97 ± 1.1[c]	0.001
10w	47.25 ± 1.33[a]	45.32 ± 1[ab]	42.63 ± 0.94[bc]	40.97 ± 1[c]	0.004

* [abc] Means in the same rows with different superscripts are significantly different at ($P<0.05$).

DISCUSSION

The stocking density is considered an important factor affecting fish welfare in the aquaculture **Ashley (2007**). Fish require sufficient space to appear nearly all normal behavior with less pain, stress and fear **FAWC (1996).** The stocking density refers to the weight of fish per unit volume or per unit volume in unit time of water flow through the holding environment. While carrying capacity refers to the maximum number of fish that an environment can provide through supplying oxygen and removal of metabolic waste products and it will be determined by the rate of oxygen consumption by fish and the response of fish to metabolic waste products such as CO_2 and ammonia (**Ellis, 2001).** The data **in Table (3)** and **Figures (1&2)** revealed that stocking density significantly affect surfacing behavior frequency and duration, where high stocking densities specially G_4 (**0.51 ± 0.45** bout and**7.75 ± 8. 43** sec) showed the highest values of surfacing frequency and duration, respectively. While low density (**0.06 ± 0.12** bout and **0.98 ± 2.10** sec) showed the minimum values of surfacing frequency and duration, respectively. The obtained results agreed with **Ellis *et al*. (2002)** who mentioned that increasing the fish biomass in a given volume of water decreases dissolved oxygen concentration, so high densities can decrease dissolved oxygen to levels below 5 mg l^{-1}. The results also agreed with **Noga (1996)** who noted that fish become near the water surface to gulp air due to low dissolved oxygen in aquarium, so increase of the surfacing behavior by fish acts as an indicator to the oxygen condition in water.

Regarding to the aggressive behavior (frequency and duration) as revealed in **Tables (4-11) and Figures (3-18)**, high stocking densities showed the highest means of frequency and duration of all aggressive patterns (approach, chasing, fin tugging, biting, butting, fleeing, mouth pushing, spreading of fins) than low and medium stocking densities as high stocking densities showed more approach, chasing, biting and fleeing activities during competition for food resources and sheltering site. While, they showed more butting, mouth pushing, spreading of fins activities for formation of dominance rank that positively related to large body size and level of aggression leading to appearance of the dominant and subordinates. These findings agreed with **Whiteman & Cote (2004)** and **Ashley (2007),** who found that high stocking culture increased competition, aggressive behavior, and physical injury (due to increasing contact between fish). Also, it confirmed that aggressive behavior is the main cause of injuries to the eyes, tails and pectoral fins causing secondary infections and mortality, and the majority of contact damaging among fish occurred during hand feeding. These findings also agreed with **Gonçalves-de-Freitas *et al.* (2019)** who stated that for social species such as Nile tilapia, the number of individuals in a group is related to the probability of encounters and due to the prediction of the larger the group is, the higher the probability of fighting so Nile tilapia shows aggressive interactions to achieve social rank and territory that is marked by biting, mouth fighting, tail beating (known as overt fight), and by signals like threats and other displays (restrained aggression).

These results also agreed with **Keeley** (**2000**) who mentioned that high stocking density can reinforce aggressive patterns among fish due to increasing aggression rates with increasing density. These findings also agreed with **Abbott & Dill (1985)** who found that attacks of dominant fish to subordinates as a result of rearing at high stocking density through biting, butting and mouth pushing directed at the body causing loss of scale, at the head leading to damaging the gills, eyes and mouthparts and at the tail, caudal and pectoral fins resulting in fin erosion, so aggressive behavior decreased the welfare status of subordinates. These results go hand by hand with that obtained by **Cole & Noakes (1980)** who found that increasing of stocking density increased the frequency and duration of aggressive patterns. Also agreed with **Wagner *et al*. (1996)** who mentioned that fighting among fish appears to end with one fish fleeing or signaling subordinate status through dark coloration, lack of activity and position within the aquarium. These results may be attributed to adverse effect of small space allowance on fish welfare. However, the reserve trend was observed by **Van de Nieuwegiessen *et al*. (2009)** who stated that high stocking density had a suppressive effect on aggressive behavior. This contrast between results may be referred to various managerial techniques.

Data in **Table (12) and Figure (19)** revealed that mid line crossing test (frequency) significantly affected by stocking density, where it was the highest in $\mathbf{G_1}$ (**2.21 ± 0.72**bout**)** and the lowest in G_4 (**0.85 ± 0.13**bout)**.** This finding attributed to at high stocking density, activity decreased with decreasing space per fish. This result goes hand by hand with the data cited by **Martins *et al*. (2012**) who found that water quality parameters affected on swimming behavior. For example, decreased dissolved oxygen levels (hypoxia) as in G_4 could decrease the swimming speeds and activity.

The data presented in **Table (13)** revealed that average body weight significantly affected by different stocking density throughout weeks of experiment, where final body weight was the greatest in $\mathbf{G_1}$ (**47.25 ± 1.33** $^{\mathbf{a}}$ g) and the lowest in G_4 (**40.97 ± 1** $^{\mathbf{c}}$ g). These results agreed with those obtained by **Boujard *et al*. (2002)** who found that high stocking density causes reductions in food intake and decrease in growth. These results agreed with **Ellis *et al.* (2002)** who stated that increasing density could influence welfare status of fish by increasing fin erosion and reduction of food intake, nutritional condition and growth. These findings also agreed with **Gonçalves-de-Freitas *et al.* (2019)** who found that high stocking density culture is directly related to welfare of Nile tilapia as it influences food competition and consumption, growth, stress, health, and mortality.

CONCLUSION

In the present study, there were several changes in the behavior of Nile tilapia due to rearing at various stocking densities, with lowering dissolved oxygen lead to changing in water quality with increase of surfacing behavior, with increasing pain, stress, fear, chasing, fin tugging, biting, butting, fleeing, mouth pushing and spreading of fins for competition to obtain food and oxygen from the surface, with decreasing body weight and poor welfare in high stocking density culture. Furthermore culture with low and medium density with lowering in surfacing behavior and aggressive behavior, high body weight, this confirm stocking density plays an important role in achieving Nile tilapia welfare.

RECOMMENDATION

It is recommended that stocking density is an essential factor used in aquaculture industry for high growth and welfare with total fish harvest in ponds.

ACKNOWLEDGMENTS

We thank the staff at the Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, Egypt, for providing materials used in this study. Also, we would like to thank the anonymous referees for their helpful comments on the manuscript.

REFERENCES

Abbott, J. C. and Dill, L. M. (1985). Patterns of aggressive attack in juvenile steelhead trout (Salmo gairdneri). Canadian Journal of Fisheries and Aquatic Sciences, 42: 1702–1706.

Altmann, J. (1974). Observational study of behavior: sampling methods. Behaviour, 49: 227-267.

APHA, American Public Health Association (1998). Standard methods for the examination of water and waste. APHA,WEF and AWWA, 20th ed, Washington Dc, USA, 11:1193.

Ashley, P. J. (2007). Fish welfare: current issues in aquaculture. Applied Animal Behaviour Science, 104:199–235.

Barton, B. A. and Iwama, G. K. (1991). Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Reviews of Fish Diseases, 1:3-26.

Bjornsson, B. (1994). Effects of stocking density on growth rate of halibut (Hippoglossus hippoglossus L.) reared in large circular tanks for three years. Aquaculture, 123: 259-270.

Boujard, T.; Labbe, L. and Auperin, B. (2002). Feeding behavior, energy expenditure and growth of rainbow trout in relation to stocking density and food accessibility. Aquaculture Research, 33:1233–1242.

Chowdhury, D. K. (2011). Optimal feeding rate for Nile tilapia .(Oreochromis niloticus) Department of Animal and Aquacultural Sciences Master Thesis 60 credits 2011, Norwegian University of Life Sciences.

Cole, K. S. and Noakes, D. L. G. (1980). Development of early social behavior of rainbow trout, Salmo gairdneri (Pisces, Salmonidae). Behavioral Processes 5: 97–112.

Ellis, T. (2001). What is stocking density? Trout News, CEFAS, 32: 35–37.

Ellis, T. ; North, B. ; Scott, A. P. ; Bromage, N. R. ; Porter, M. and Gadd, D. (2002). The relationships between stocking density and welfare in farmed rainbow trout. Journal of fish biology, 61:493–531.

El-Sayed, A. F. M. (2006). Environmental requirements in Tilapia Culture, pp. 34-46. CABI Publishing, Walling-ford, Oxfordshire, UK.

Fall, F. M. (2005). Lab exercise: Techniques for behavioral research in guppies bowling green state university: Animal behavior – biology, 420/543: 1-5.

FAWC, Farmed Animal Welfare Council (1996). Report on the Welfare of Farmed Fish. Surbiton, Surrey.

Ferey, D. F. and Miller, R. j. (1972). The establishment of dominance relationships in the blue gourami. Behaviour volume XLII - parts (1-2): 9-59.

Gonçalves-de-Freitas, E. ; Bolognesi, M. C. ; Gauy, A. C. D. S. ; Brandão, M. L. ; Giaquinto, P. C. and Fernandes-Castilho, M. (2019). Social Behavior and Welfare in Nile Tilapia. Fishes, 4: 23.

Keeley, E. R. (2000). An experimental analysis of territory size in juvenile steelhead trout. Animal Behavior, 59: 477–490

Khalil, A.; Husseiny, W.; Fattah, A. and Ghonimi, W. (2016). Effect of feeding with different dietary protein levels and starvation on the health, nonspecific immune parameters, behavior and histo-architectures of fantail goldfish (Carassius auratus L.). Journal of Veterinary, Science and Technology, **7**: 2-12.

Martins, C. I.; Galhardo, L.; Noble, C.; Damsgård, B.; Spedicato, M. T.; Zupa, W. ; Beauchaud, M.; Kulczykowska, E. Massabuau, J. C. and Carter, T. (2012). Behavioral indicators of welfare in farmed fish. Fish Physiology and Biochemistry, 38: 17-41.

Noga, E. J. (1996). Fish Diseases Diagnosis and Treatment. First edition, Mosby electronic publishing.

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

Osofero, S.; Otubusin, S. and Daramola, J. (2009). Effect of stocking density on tilapia (Oreochromis niloticus Linnaeus 1757) growth and survival in bamboo–net cages trial. African Journal of Biotechnology, 8 (7): 1322-1325.

Pavanelli, G. C. ; Eiras, J. C. and Takemoto, R. M. (2008). Doenças de Peixes: profilaxia, diagnóstico e tratamento. 3ª ed. p. 311 (ed by Eduem).

Salama, M. E. A.; Moustafa Y.T.; El-Dahhar, A. A. and Dawah, A. M. (2006). Effect of fertilization on production of Nile tilapia in earthen ponds. II) Effect of an untraditional organic fertilizer and stocking density on the fish yield of mixed-sex Nile tilapia (Oreochromis niloticus). J Arab Aquacult Soc, 1: 112–130.

Salas, L; Anguis, V.; Martin, A, B.; Crespo, D.; Planas, J.V.; Infante, C.; canavate, J. P. and Manchado, M. (2010). Effects of stocking density and feed ration on growth and gene expression in the Senegalese sole (Solea senegalensis): Potential effects on the immune response. Fish and Shellfish Immunology, 28(2): 296-302.

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

Scheurmann, I. (2000). The Natural Aquarium Handbook. Second edition, Baron Educational Series Inc. Hauppauge, N.Y.

Schreck, C. B.; Olla, B. L. and Davis, M. W. (1997). Behavioural response to stress. In: Iwama, G.; Pickering, A.; Sumpter, J.; Schreck, C. (Eds.) Fish Stress and Health in Aquaculture. Cambridge University Press, Cambridge, pp. 145–170.

Scott, G. R.; Sloman, K. A.; Rouleau, C. and Wood, C. M. J. J. O. E. B. (2003). Cadmium disrupts behavioural and physiological responses to alarm substance in juvenile rainbow trout (Oncorhynchus mykiss). Journal of Experimental Biology, 206: 1779-1790.

Silva, P. C.; Souza, V. L.; Padua, D. M. C.; Dalacorte, P.C. and Goncalves, D.C. (2000). Effect of stocking density on growth and fillet composition of tetra hybrid red tilapia, Israeli strain. In: K. Fitzsimmons and J.C. Filho (Eds.). Tilapia Aquaculture in the 21st Century. Proceedings from the 5th International Symposium on Tilapia Aquaculture. Rio de Janeiro, Brazil. 2: 341-345.

SPSS version 21 (2012): IBM Corp. IBM SPSS Statistics for Windows, Armonk, and NY.

Srinivasan, P.; Darsini, D.T.P.; Maheshu, V.; Castro, J.; Dineshbabu, J. and Manimekalai, K. (2015). Limonia acidissimal. (Wood apple) as feed additive enhanced growth performance, immune response and disease resistance of Indian major carp (Catla catla) against aeromonas hydrophila infection. International Research Journal of Pharmacy, 6 (2): 143-152.

Van de Nieuwegiessen, P. G.; Olwo, J.; Khong, S.; Verreth, J. A. J. and Schrama, J. W. (2009). Effects of age and stocking density on the welfare of African catfish, Clarias gariepinus Burchell. Aquaculture, 288 (1-2): 69–75.

Wagner, E. J.; Intelmann, S. S. and Routledge, M. D. (1996). The effects of rearing density on hatchery performance, fin condition, and agonistic behavior of rainbow trout Oncorhynchus mykiss fry. Journal of the World Aquaculture Society, 27: 264–274.

Whiteman, A. E. and Cote, I. (2004). Dominance hierarchies in group-living cleaning gobies causes and foraging consequences. Animal behavior, 67: 239-247.