Dietary Cholesterol and Aggression in Nile tilapia Oreochromis niloticus

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Abstract

Tilapia farmers would benefit tremendously if they could decrease aggression among fish. Conspecific aggression affects growth, feed conversion and general wellbeing of fish. Previous studies established an inverse relationship between blood cholesterol levels and aggression in fish, whereby a decrease in cholesterol led to an increase in aggression. The present study assessed the effect of an increase of dietary cholesterol on blood cholesterol and possible decrease in aggression of Nile tilapia *Oreochromis niloticus*. Nile tilapia were stocked in an outdoor recirculation system then offered one of five diets: 0% cholesterol, 0.5% cholesterol, 1% cholesterol, 1.5% cholesterol and 2% cholesterol. Five fish of each treatment were moved to a glass tank and monitored for signs of aggression for 10 minutes, twice a day. This experimental procedure was repeated five times using a new set of fish every time. Results show an increase in cholesterol levels in the blood but that there are no significant differences in aggression among treatments. Accordingly, the present study suggests that an increase in dietary cholesterol increases blood cholesterol in fish but does not have a significant effect on antagonistic patterns in Nile tilapia.

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Tilapia farmers would benefit tremendously if they could decrease aggression among fish. Conspecific aggression affects growth, feed conversion and general wellbeing of fish. Previous studies established an inverse relationship between blood cholesterol levels and aggression in fish, whereby a decrease in cholesterol led to an increase in aggression. The present study assessed the effect of an increase of dietary cholesterol on blood cholesterol and possible decrease in aggression of Nile tilapia *Oreochromis niloticus* . Nile tilapia were stocked in an outdoor recirculation system then offered one of five diets: 0% cholesterol, 0.5% cholesterol, 1% cholesterol, 1.5% cholesterol and 2% cholesterol. Five fish of each treatment were moved to a glass tank and monitored for signs of aggression for 10 minutes, twice a day. This experimental procedure was repeated five times using a new set of fish every time. Results show an increase in cholesterol levels in the blood but that there are no significant differences in aggression among treatments. Accordingly, the present study suggests that an increase in dietary cholesterol increases blood cholesterol in fish but does not have a significant effect on antagonistic patterns in Nile tilapia.

Keywords: Nile tilapia, aggression, dietary cholesterol, antagonistic behavior.

Introduction

Nile tilapia is a territorial fish whose social interactions are hierarchical. The position/ rank of the fish in the hierarchy is established through aggressive displays and can grant fish better access to food (Hodapp & Frey, 1982; Magnuson, 1962; Metcalfe, 1986; Ward, Webster, & Hart, 2006), mates (Fricke, 1979; Parzefall, 1969) and territory (Barlow, 2002; Huntingford & Turner, 2013). As such, when fish of various sizes occupy the same niche, the dominant Nile tilapia grows more than subordinates because of differences in feed acquisition, digestion rate, and energy expenditure (Gonçalves-de-Freitas et al., 2019), with economic costs to the farmer.

In aquaculture, fish are often size sorted before stocking to ensure optimum growth (Saoud, Davis, Roy, & Phelps, 2005; Ghanawi, Shalabi & Saoud 2010) and equal access to resources. The problem is that fish grading allows unfamiliar fish of similar fighting ability to be sorted together (Slavík, Pešta, & Horký, 2011). Fish of similar sizes tend to have prolonged and escalated fights (Boscolo, Morais, & Goncalves-de-Freitas, 2011; Enquist & Jakobsson, 1986) that result in harm to the individuals involved. Moreover, aggressive interactions among fish increase the metabolic cost and energy expenditure of fish and trigger social stress thus affecting fish health (Carvalho, Mendonça, Costa-Ferreira, & Gonçalves-de-Freitas, 2013; Gonçalves-de-Freitas et al., 2019; Metcalfe, 1986). Because stress is a major cause of morbidity and mortality in aquatic systems (Conte, 2004), farmers try to manage stress to improve fish welfare.

Cholesterol is a zoosterol that has important structural and biochemical roles in the body. For instance, cholesterol is an important constituent of cell membrane and plasma lipoproteins as well as a precursor of various steroid hormones (Myant, 1973). Teleost fish, like other vertebrates, are capable of synthesizing cholesterol *de novo*(Leaver et al., 2008) and thus don't require it in their diets. In 2018, Aguiar and Giaquinto (2018) treated Nile tilapia with a cholesterol-lowering statin and noticed that low plasma cholesterol was associated with an increase in aggression in the fish. Results of the study corroborated the findings of previous studies performed on primates (Fontenot, Kaplan, Shively, Manuck, & Mann, 1996; Kaplan, Manuck, & Shively, 1991; Kaplan et al., 1994) reporting an inverse relationship between blood cholesterol levels and aggression. In the present study we propose a twist on Aguiar and Giaquinto (2018) where we incorporate cholesterol in the diets of Nile tilapia, and test whether dietary cholesterol mitigates antagonistic interactions in the fish. We investigated whether an increase in dietary cholesterol is associated with an increase in blood cholesterol in Nile tilapia, and whether that increase is inversely correlated with aggression.

Materials and Methods

Fish Maintenance

The present study was performed at the aquatic physiology laboratory of the American University of Beirut (AUB), Lebanon (August 2020- October 2020). Animal welfare and use protocols were approved by the IRB at the university. Data can be obtained from the author upon demand. Nile tilapia used in the present experiment were spawned in outdoor 1 m^3 circular tanks in a recirculation aquaculture system. Swim-up fry were collected, stocked in 180-L fiberglass tanks of a recirculation system, and sex-reversed using α-methyl testosterone in the feed. Sex-reversed fry were then transferred to the outdoors recirculation system and offered a 40% crude protein, 6% lipid commercial feed (Rangen Inc., Buhl, Idaho, USA) twice daily to apparent satiation, until used in the experiment.

Treatments

Five isonitrogenous and isolipidic feeds were prepared containing increasing concentrations of cholesterol. Treatments were (T1): 0% cholesterol, (T2): 0.5% cholesterol, (T3): 1% cholesterol, (T4): 1.5% cholesterol and (T5): 2% cholesterol (Table 1). Ingredients were mixed with hot water and extruded in a meat grinder through a 2mm die then dried in a forced-air oven at 45°C until constant weight.

Monitoring aggression

Fish used in the present experiment were acclimated in five 1 m^3 outdoor holding tanks connected to a biological filter in a recirculation system. Water was aerated using submerged air diffusers connected to a regenerative blower. Nile tilapia were size sorted by hand, and 34 fish (46.0 ± 14.97 g; mean ± SD) were stocked in each tank. Each tank was assigned one of the five dietary treatments and fish were offered feed at 3% body mass (BM) divided into two daily portions, at 8:00 and 17:00 hours, for two weeks. At the end of the two weeks, a subset of the fish in each tank were relocated from the holding tanks to 52-L glass tanks connected to a biological filter, and a sand filter in a recirculation system, to monitor aggression. Five fish of similar sizes were randomly collected from each of the five holding tanks, group-weighed and stocked in the glass tanks. Fish were left to acclimate in the glass tanks for 24 hours, and offered their respective feeds at 3% BM, twice daily, at 8:00 and 17:00 hours. Fish were then monitored for signs of aggression using an adaptation of the Falter (1983) ethogram. Aggressive behavior such as nipping, mouth fighting/ attack, lateral fighting/ attack, chasing and flight, and cornering were quantified twice daily for 10 minutes (after morning feeding and before second feeding) on days 2, 3 and 5 after stocking. All observations were recorded by two people and their observations compared. Number of animals per tank hindered the quantification of aggressive behavior, therefore frequency of aggressive behavior was not considered but rather the presence or absence of aggressive behavior. Data collected was used to calculate an aggression index whereby the presence of a criterion elicits a score of "1", and the absence a score of "0". As such, each tank was given a score (on a scale from 0 to 5) and average scores of three monitoring days were recorded. Observers also monitored position of fish in the tank and coloration of fish. The monitoring procedure was replicated five times using new fish from the holding tanks (Table 2). Fish stocked in previous experimental weeks continued to be observed throughout the duration of the experiment but only data collected on days 2, 3 and 5 were used to calculate the aggression index.

Serum cholesterol levels

Three weeks after stocking, five fish were randomly removed from each holding tank and blood was collected from their caudal arch using heparinized 1 mL syringes and 25-gauge needles. Plasma cholesterol levels were determined using a hand-held Accutrend Plus Meter and Accutrend Cholesterol test strips (Roche diagnostics, Risch-Rotkreuz, Switzerland).

Statistical Analysis

Data was analyzed using SAS (V.9.2, SAS Institute Inc., Cary, North Carolina, USA). All data were reported as mean values ± standard deviation of the mean and compared using one-way ANOVA. Student Newman-Keuls (SNK) mean separation test was used to assess significant differences among means. Significance level was set at $p < 0.05$. Original research data are not shared but can be made available upon request.

Results

Results of the present experiment showed that plasma cholesterol in fish offered any of the cholesterol-enriched diets was greater than plasma cholesterol in control fish. All fish in the various treatments had cholesterol levels greater than detection limits (Table 3). Furthermore, there were no significant differences in aggression among any of the treatments (Table 4). In all treatments, observers were able to identify the dominant Nile tilapia characterized by a bright coloration, instigation of attacks and display of the dorsal fin (Giaquinto & Volpato, 1997). Alternatively, subordinate fish had a dark coloration or dark stripes that got darker after confrontation with dominant fish. Similarly, in all treatments dominant tilapia were observed to be swimming freely whilst subordinates often grouped in a corner of the tank. Displays of dominance were observed across treatments, whereby the dominant fish were gulping and jotting their heads frequently. There were no significant differences in mortality among treatments.

Discussion

Supplementing tilapia diets with cholesterol results in an increase in plasma cholesterol levels but does not decrease aggression among the fish. In the Aguiar and Giaquinto (2018) study, aggression of fish with 200 mg/dL plasma cholesterol was less than that of fish with 400 mg/dL. However, in the present study fish with 187 mg/dL cholesterol were just as aggressive as those with plasma cholesterol greater than 300 mg/dL.

Previous studies investigating the relationship between cholesterol and aggression often attributed increased aggression to a decrease in dietary cholesterol (Fontenot et al., 1996; Kaplan et al., 1991; Kaplan et al., 1994). However, Kaplan et al. (1994) did mention that observed results could actually be caused by increased dietary cholesterol suppressing antagonistic behavior. It is interesting to note that in batch 3, and especially in treatments 4 (1.5% cholesterol) and 5 (2% Cholesterol), the dominance hierarchy became vague. In fact, observers noticed the emergence of another fish that had similar characteristics to the dominant fish, was initiating attacks on other fish and even confronting the dominant. However, we couldn't find a clear explanation to such behavior.

It is important to note that the observed fish were placed in a recirculating system and water was renewed constantly to remove feces and excess feed. Accordingly, observed results could be caused by water renewal diluting the chemicals needed for conspecific recognition and thus destabilizing the hierarchy (Gonçalves-de-Freitas, Teresa, Gomes, & Giaquinto, 2008). Another possible explanation for present results could be that cholesterol effects on aggressiveness obeyed the rules of saturation kinetics and when concentrations became greater than a specific threshold, effects were no longer observed. No matter the mechanism involved, adding cholesterol to tilapia diets did not decrease antagonistic behavior and thus confers no benefit to farmers.

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Tables

Table 1. Ingredients of the five diets offered to *Oreochromis niloticus* for five weeks

Ingredient	Diet 1	Diet 2	Diet 3	Diet 4	Diet
	0% Cholesterol	0.5% Cholesterol	1% Cholesterol	1.5% Cholesterol	2% C
Fishmeal [a] (g/ Kg)	200	200	200	200	200
Soybean meal solvent extracted [b] (g/ Kg)	450	450	450	450	450
Soy oil (g/ Kg)	35	30	25	20	15
Wheat flour (g/ Kg)	30	30	30	30	30
Whole Wheat (g/ Kg)	241	241	241	241	241
Vitamin & mineral premix [c] (g/ Kg)	20	20	20	20	20
Choline chloride (g/ Kg)	5	5	5	5	5
Stay C 250 [d] (g/ Kg)	1	1	1	1	1
CaP-dibasic (g/ Kg)	2	2	2	2	2
Cholesterol [e] (g/ Kg)	0	5	10	15	20
DL-Methionine (g/ Kg)	1	1	1	1	1
Gelatin [f] (g/ Kg)	15	15	15	15	15
Total (g)	1000	1000	1000	1000	1000

a: FF Skagen Denmark. Havnevagtvej 12.9990 Skagen.

b: De-hulled solvent extracted soybean meal, Southern Sates Cooperative Inc., Richmond, VA, USA.

c: The vitamin and mineral premix provided the following per kg of experimental diet: vitamin A retinyl acetate 1 million IU, vitamin D3 cholecalciferol 0.1 million IU, vitamin E alpha-tocoph acet 7 g, vitamin K 0.5 g, folic acid niacin 0. 1 g, niacin 4 g, calcium pantothenate 2.5 g, riboflavin (B2) 0.6 g, vitamin B12 0.001 g, thiamine (B1 nitrate) 0.5 g, pyridoxine (B6 HCl) 0.5 g, biotin 0.0125 g, vitamin C (ascorbic acid) 0.25 g, inositol 5 g, selenium (as sodium selenite) 0.0045 g, iodine (as calcium iodate) 0.25 g, iron (as sulphate monohydrate) 2 g, zinc (as oxide) 5 g, copper (as sulphate pentahydrate) 0.25 g, manganese (as sulphate monohydrate) 3.5 g, chlorine chloride 75, phosphorus (as monodicalcium phosphate) 2.5, sodium chloride (salt) 225 g, and cellulose 75 g. Calcium carbonate carrier to balance.

d: 250 mg kg - 1 active vit C supplied by Stay C®, (L-ascorbyl-2-polyphosphate 25% Active C), Roche Vitamins Inc., Parsippany, NJ, USA.

e: USP cryst. From Lanolin. Fluka AG, CH-9470 Buchs.

f: Himedia laboratories Pvt. Ltd., 23, Vadhani Ind. Est., LBS Marg, Mumbai, India.

Table 2. Stocking and monitoring schedule of Nile tilapia,

Oreochromis niloticus.

Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
S1													
						S2							
										S3			
												S4	

S: Stocking

: monitoring time 10 minutes/ tank

: monitoring time 5 minutes/ tank

: no monitoring

Table 3. Group weights (g) of *Oreochromis niloticus* stocked in the glass tanks for observation of antagonistic behavior.

Diet	Treatment	Weight (g)	Weight (g)	Weight (g)	Weight (g)	Weight (g)
		Batch 1	Batch 2	Batch 3	Batch 4	Batch 5
1	0 % Cholesterol	320.7	390.6	289.9	379.5	349.5
2	0.5 % Cholesterol	280.0	428.0	306.8	275.9	388.2
3	1 % Cholesterol	263.4	355.4	293.5	474.8	346.9
4	1.5 % Cholesterol	252.4	335.3	308.2	286.0	423.1
5	2 % Cholesterol	254.0	353.8	322.9	298.0	284.3

Table 3. Serum cholesterol levels (mg/dL) of *Oreochromis niloticus* offered various percentages of cholesterol in their diets.

Diet	Treatment	Serum cholesterol (mg/dL)
1	0% Cholesterol	187
2	0.5% Cholesterol	Hi* (¿ 300)

Diet	Treatment	Serum cholesterol (mg/dL)
3	1% Cholesterol	Hi (¿ 300)
4	1.5% Cholesterol	Hi (¿ 300)
5	2% Cholesterol	Hi (¿ 300)

*Hi: Cholesterol measured in the sample exceeded the detection limit of Accutrend Plus Meter (Detection limit Lo ¡150 mg/dL-Hi ¿300 mg/dL).

Table 4. Aggression indices of Nile tilapia offered various percentages of dietary cholesterol.

Diet	Treatment	Day 2	Day 3	Day 5
1	0% Cholesterol	2.75 ± 0.50^{a}	3.06 ± 0.31^{a}	3.00 ± 0.41^{a}
2	0.5% Cholesterol	3.25 ± 0.50^{a}	3.04 ± 0.34^{a}	2.50 ± 1.22^{a}
3	1% Cholesterol	2.70 ± 0.91^{a}	2.72 ± 1.15^{a}	2.50 ± 0.94^{a}
4	1.5% Cholesterol	2.70 ± 0.45^{a}	3.78 ± 0.70^{a}	2.70 ± 0.57^{a}
5	2% Cholesterol	2.80 ± 0.67^{a}	2.43 ± 0.85^{a}	3.50 ± 0.79^{a}

Data was reported as mean ± standard deviation.

Values in the same column with different superscripts are significantly different from each other ($p < 0.05$).