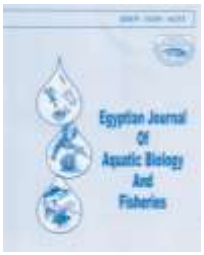
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 - 6131 Vol. 22(3): 25- 36 (2018)
ejabf.journals.ekb.eg



Effect of lipid feeding regimes and conditioning periods on gonads fatty acid profile and reproductive performance of the Nile tilapia (*Oreochromis niloticus)* broodstock

Osama M. El-Husseiny[1]; Ashraf M. A.-S. Goda[2]; Rania S. Mabroke*[1] and Ali Matter[2]

1- Department of Animal Production, Fish Nutrition Laboratory (FNL), Faculty of Agriculture, Cairo University, Egypt
2- Fish Nutrition Research Laboratory, National Institute of Oceanography and Fisheries (NIOF), Egypt
*Corresponding author: Rania.Ahmed@agr.cu.edu.eg, raniasms2002@yahoo.com

ARTICLE INFO
Article History:
Received:May 12, 2018
Accepted:June 28, 2018
Available online:July 2018

Keywords:
Nile tilapia
Oreochromis niloticus
Reproductive performance
Gonads
fish oil
soybean oil
feeding regimes
conditioning period

ABSTRACT

Under condition of hapa-in-pond system, different lipid feeding regimes and conditioning periods were examined for their effect on fatty acid profile of tilapia female ovaries; subsequently their reproductive performance. Three diets were formulated; soybean oil-based diet (SBO), fish oil-based diet (FO) or diet containing fish oil:soybean oil (1:1; MIX). The diets were used to perform five feeding regimes; FO diet continuously (FOcont.), SBO diet continuously (SBOcont.), Mix diet continuously (MIXcont.), FO in the morning then SBO in the afternoon (FOam/SBOpm) and SBO in the morning then FO in the afternoon (SBOam/FOpm). Treatments were examined under conditioning period for two and four weeks. Ovaries of females fed SBOcont, MIXcont. and SBOam/FOpm showed higher values of total n-6 and n-6/n-3 ratio and the best reproductive performance. The opposite trend was noticed for females fed FOcont., and FOam/SBOpm diets. Ratio of n-6/n-3 for fish fed SBOcont., MIXcont. and SBOam/FOpm diets reached recommended ratio for the reproductive performance after two weeks of conditioning period. Ovaries accumulate fatty acids in morning more than afternoon period. Optimal reproductive performance of tilapia showed a need for FO to reach optimal ovaries n-6/n-3 ratio in short period. Delivering FO in the morning may guarantee the maximum utilization of such expensive source.

INTRODUCTION

Globally, tilapia production comes after carp, if considering the farmed quantities, meanwhile it is the main produced species in Egypt (FAO, 2010, 2012). Thus, fry of high quantity and quality are required for stocking the grow-out systems in parallel with the rapid rise in tilapia production (Wang, 2014). Nutrition of broodstock is one of the factors that influence the efficiency of seeds production (Izquierdo *et al*., 2001). Generally, gonadal maturation and broods fecundity directly rely on the quality of broodstook diets.

Egg yolk is the main source of nutrients for the embryonic development stages, despite the ability to absorb some nutrients directly from water (Mabroke *et al*., 2012). Fecundity, inter-spawning intervals, hatchability, egg viability and larvae growth are found to be greatly affected by the lipid and fatty acid profile of broodstock diets (Fernández-Palacios *et al*., 1995; Izquierdo *et al.,* 2001).

Tilapia broodstock have more needs for dietary n-6 HUFA than n-3 HUFA series and it was suggested that a balanced mixture between vegetable and fish oil sources is needed for optimal reproductive performance (Santiago and Reyes, 1993; El-Sayed *et al*., 2005; Hajizadeh *et al.,* 2008; Ng and Wang, 2011). Upon our knowledge, no researcher work investigated the effect of time of the day on lipid sources and fatty acid utilization of tilapia broodfish. Feeding time may influence a number of physiological variables including weight gain and fattening as aquatic animals display nature behavioral rhythms (López-Olmeda *et al.*, 2012). Nile tilapia (*Oreochromis niloticus*) utilized feed more efficiently during afternoon period and exhibited better growth performance (El-Husseiny *et al*., 2004).

Conditioning of tilapia broodstock for a period of time before the spawning is another factor beside efficient lipid utilization that has been reported as an important tool to improve the reproductive performance (Lovshin and Ibrahim, 1988; Costa-Pierce and Hadikusumah, 1995; Bhujel, 2000; El-Sayed, 2006; Abou-Zied, 2015). Conditioning or resting of fish is known as maintaining of both females and males separately in different units, for a period of time before and between spawning, at a high stocking density, with good feeding regimes (Bhujel, 2000). Some authors suggested that conditioning of tilapia broodstock for short period (5-16 days) is optimal than long period (21-30days) (Costa-Pierce and Hadikusumah, 1995; Bhujel, 2000; El-Sayed, 2006) while, Lovshin and Ibrahim (1988) suggested that tilapia broodstock rested for 21 days showed better reproductive performance than non-rested broodstock. Very limited information is available about the effect of conditioning period on Fatty acids (FA) profile of tilapia gonads. Therefore the aim of the study was to investigate the effect of conditioning period and lipid feeding regimes on the fatty acid profile of tilapia ovaries, subsequently reproductive performance under condition of hapa-in-pond system.

MATERIALS AND METHODS

Experimental Tilapia broodstock diets:

Three diets with isonitrogenous (35.9%) and isolipidic (9.7%) were formulated (Table 1). The formulated diets exhibited different fatty acid profiles (Table 2) as soybean oil-based diet (SBO), fish oil-based diet (FO) or diet containing mixture of FO and SO 1:1 (MIX). The dry ingredients were mixed with oil and water manually. The dietary dough was pressed to form pellets. Pellets were air-dried, manually broken down into small crumbles and stored at (4ºC) till use.

Conditioning period of Tilapia broodstock (pre spawning):

This study was conducted at El-Qanater Fish Research Station, Qaliubiya Governorate, National Institute of Oceanography and Fisheries (NIOF), Egypt. Conditioning period lasted for four weeks. Fish were obtained from local hatchery, Kafr El-shaikh governorate, Egypt. They were sexed and stocked for two weeks for acclimatization purpose before the start of the experiment. Number of 110 females with an average weight of 55±1g and 40 males with an average weight of 52±1g of Nile tilapia (*Oreochromis niloticus*) were used.

Table 1: Ingredient and proximate composition of the experimental diets.

Ingredient (g/kg)	*FO*	*SBO*	*MIX*
Fish meal[a]	250	250	250
Soybean meal[b]	350	350	350
Corn[c]	314.4	314.4	314.4
Fish oil[d]	60	0	30
Soybean oil[e]	0	60	30
Premix[f]	20	20	20
Vitamin C[g]	0.5	0.5	0.5
CMC[h]	5	5	5
BHT[i]	0.1	0.1	0.1
Total (g)	1000	1000	1000
Proximate composition			
Moisture%	7.7	8	7.9
Protein%	35.9	35.8	35.9
Lipid%	9.7	9.6	9.7
Ash%	9.7	9.6	9.6
Total carbohydrates%[j]	44.7	45	44.8
Gross energy (kcal/kg)[k]	4804.45	4802	4808.65

[a] Danish fish meal 72% crude protein.
[b] Soybean contains 42% crude protein.
[c] Yellow corn containing 9.6% crude protein.
[d] Imported fish oil.
[e] Imported soybean oil.
[f] Provides per kg of diet: retinyl acetate, 3,000 IU; cholecalciferol, 2,400 IU; all-rac-α-tocopheryl acetate, 60 IU; menadione sodium bisulfite, 1.2 mg; ascorbic acid monophosphate (49 % ascorbic acid), 120 mg; cyanocobalamine, 0.024 mg; d-biotin, 0.168 mg; choline chloride, 1,200 mg; folic acid, 1.2 mg; niacin, 12 mg; d-calcium pantothenate, 26 mg; pyridoxine. HCl, 6 mg; riboflavin, 7.2 mg; thiamin. HCl, 1.2 mg; sodium chloride (NaCl, 39 % Na, 61 % Cl), 3,077 mg; ferrous sulfate ($FeSO_4 \cdot 7H_2O$, 20 % Fe), 65 mg; manganese sulfate ($MnSO_4$, 36 % Mn), 89 mg; zinc sulfate ($ZnSO_4 \cdot 7H_2O$, 40 % Zn), 150 mg; copper sulfate ($CuSO_4.5H_2O$, 25 % Cu), 28 mg; potassium iodide (KI, 24 % K, 76 % I), 11 mg; Celite AW521 (acid-washed diatomaceous earth silica), 1,000 mg Agri-Vet Co., Cairo, Egypt.
[g] Ascorbic acid.
[h] Carboxy-methyl cellulose.
[i] Butylated hydroxyl toluene.
[j] Total carbohydrate content was determined by the difference: total carbohydrate = 100 − (% crude protein + % crude fat + % total ash + % Moisture).
[k] Dietary gross energy was calculated using the conversion factors of 5.6, 9.45 and 4.2 kcal/kg for protein, lipids and carbohydrates, respectively.

Table 2: Fatty acid composition (% total fatty acids) of experimental tilapia broodstock diets supplemented with different lipid sources.

Fatty acids	*FO*	*SBO*	*MIX*
C14:0	2.67	ND[a]	1.34
C16:0	9.73	13.1	11.41
C17:0	7.9	ND	3.95
C18:0	2.71	4.57	3.64
C20:0	0.65	0.88	0.77
C22:0	ND	1.18	0.59
∑SFA[b]	23.65	19.72	21.69
C14:1	3.09	0.49	1.79
C16:1	5.81	1.22	3.52
C17:1	1.09	ND	0.54
C18:1 n-9	13.33	26.81	20.07
C20:1	ND	0.73	0.37
C22:1 n-9	0.34	3.33	1.83
∑MUFA[c]	23.66	32.58	28.12
C18:2 (n-6)-t	2.96	ND	1.48
C18:2 n-6 (LA)	24.83	43.43	34.13
C18:3 n-3 (ALA)	1.34	0.76	1.05
C18:3 (n-6)-γ	1.97	ND	0.99
C20:3 n-3	7.03	0.36	3.7
C20:4 n-6 (ARA)	7.5	1.17	4.34
C20:5 n-3 (EPA)	0.49	ND	0.24
C22:6 n-3 (DHA)	6.56	1.97	4.26
∑MC-PUFA[d]	31.1	44.2	37.65
∑LC-PUFA[e]	21.58	3.5	12.54
∑PUFA[f]	52.69	47.69	50.19
∑n-3[g]	15.42	3.09	9.26
∑n-6[h]	37.26	44.6	40.93
n-6 : n-3	2.42	14.43	4.42
PUFA : SFA	2.23	2.42	2.31

[a] ND, not detected.
[b] Total SFA, total saturases, included 14:0, 16:0, 17:0, 18:0, 20:0 and 22:0 .
[c] Total MUFA, total monoenes, included 14:1, 16:1, 17:1, 18:1 n-9, 20:1and22:1 n-9.
[d] Medium-chain PUFA, sum of all PUFA with chain length of 18 carbon atoms.
[e] Long-chain PUFA, sum of all FA with chain length≥20 carbon atoms and ≥3 double bonds.
[f] Total PUFA included 18:2 (n-6)-t, 18:2 n-6, 18:3 n-3, 18:3(n-6)-γ, 20:3 n-3, 20:4 n-6, 20:5 n-3 and 22:6 n-3.
[g] Total n-3 included 18:3 n-3, 20:3 n-3, 20:5 n-3 and 22:6 n-3.
[h] Total n-6 included 18:2(n-6)-t, 18:2 n-6, 18:3(n-6)-γ and 20:4 n-6.

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distributed separately into two hapas (20 each) in the same cement pond. Either females or males fed at 10:00 and 16:00 to apparent satiation by hand. Females were fed on the experimental diets using different lipid feeding regimes which are described in Table 3 while, males fed SBO diet throughout the conditioning period. Continuous aeration was employed for all the breeding hapas. Quality parameters of water including temperature, dissolved oxygen, pH and total ammonia of the experimental water were monitored weekly (Table 4) using (Professional Plus, YSI 1, USA) while, fish weight was detected biweekly.

Reproductive performance of Tilapia broodstock:

After 4wks of conditioning period, fish of each lipid feeding regime (two replicate) were pooled and re-distributed into triplicate in conventional breeding hapas (135×190×50 cm) placed in a cement pond (80 m^3). Each hapa was stocked with sex ratio 2 : 1 (four females, mean weight, 93.08±1.14 g, and two males, mean weight, 86.5±1.44 g). The previous five different experimental lipid feeding regimes (Table 3) which were applied throughout the conditioning period were continuously tested for 39 days, throughout the reproductive period, broodstock fed at 10:00 and 16:00 to apparent satiation by hand. Females were weighted and checked for spawning activity (eggs or fry in buccal cavity) biweekly. For 39 days, seeds were collected and counted (two batches, after 14 days and 25 days).

Table 3: Design of different lipid feeding regimes that used throughout the experimental period.

Treatments assigns	*Feeding regimes*
$FO_{cont.}$	Fish fed FO diet continuously.
$SBO_{cont.}$	Fish fed SBO diet continuously.
$MIX_{cont.}$	Fish fed MIX diet continuously.
FO_{am}/SBO_{pm}	Fish fed FO at 10:00 then fed SBO at 16:00.
SBO_{am}/FO_{pm}	Fish fed SBO at 10:00 then fed FO at 16:00.

Reproductive performance parameters:

Absolute fecundity, relative fecundity and system productivity were calculated as the following: Absolute fecundity = Mean number of seeds at each spawning per female. Relative fecundity = Mean number of seeds at each spawning per female body weight (g). System productivity =Mean number of seeds per day/hapa size.

Sampling and chemical analysis:

Samples of females or males were randomly collected; at the beginning, after 2wks and after 4wks of conditioning period (two fish each), this fish as well as diets samples were frozen (-20 ºC) till subsequent chemical analysis for fatty acid profile determination. Gonado-somatic index (GSI) were determined using the following equation; Gonado-somatic index (GSI) = [gonads weight (g)/somatic weight (g)]*100

Fatty acid analysis:

Ovary samples (two ovaries each) were collected after 2 and 4 weeks of conditioning period and were chemically analyzed for detecting their fatty acid profile. Lipid extraction and preparation of fatty acids methyl ester were performed according the method of **Radwan (1978)** and **Harold *et al*. (1981)** using the HPLC apparatus in the central labs, High Institute of Public Health, Alexandria University, Egypt.

Statistical analysis:

SPSS v16.0 program were used to analyze the obtained data by one-way analysis of variance while, two ways analysis of variance were used to determination

the effect of time on the GSI data. Odd replicate values were omitted for data integrity. Data that showed significant differences at level of 0.05 were ranked using Duncan's multiple range tests.

RESULTS AND DISCUSSION

Water quality:

Water quality parameters during experimental period are shown in Table 4. All the water quality parameters were within the acceptable range for Nile tilapia (Chervinski,1982; Popma and Lovshin, 1996; Popma and Masser, 1999). Water temperature ranged from 26.9 to 30.2 °C, dissolved oxygen (DO) from 4.04 to 6.58 mg/L, pH from 8.02 to 8.75 and total ammonia nitrogen from 0.98 to 2.72 mg/L, respectively.

Table 4: Average values and range of water quality parameters during the experiment.

Parameters	*Average (low-high)*
Temperature (º C)	28.35 (26.9-30.2)
Dissolved oxygen (ppm)	5.10 (4.04-6.58)
pH	8.21 (8.02-8.75)
Total ammonia-nitrogen (mg /L)	1.36 (0.98-2.72)

Fatty acid composition of female gonads:

Fatty acid profiles of tilapia female ovaries fed under different lipid regimes for two and four weeks are showed in Tables 5 and 6. No significant differences were recorded for total saturated fatty acids (SFA) and total mono-unsaturated fatty acids (MUFA) among broodstock ovaries in different treatments after conditioned for two weeks (Table 5). Same trend was observed after four weeks period (Table 6) with some exceptions, where fish fed FO_{cont} diet had the highest concentrations of total SFA, while the lowest value was obtained for fish fed SBO_{am}/FO_{pm} diet. SFA and MUFA are known to be preferred substrates for mitochondrial β-oxidation for energy production in fish (Henderson, 1996), this may explain the insignificant effect of dietary lipid regimes on their levels in ovaries. Rodriguez *et al.* (1993) suggested the same hypothesis but for these deposited in muscles and eggs of gilthead sea bream broods.

Females fed SBO based-diets in forms $SBO_{cont.}$, $MIX_{cont.}$ and $SBO_{am}/FO_{pm}$for two weeks had the highest levels of linoleic acid (C18:2 n-6), total MC-PUFA and total n-6 on numerical basis. The last feeding regimes showed the highest n-6/n-3 ratio ($P < 0.05$) and the best reproductive performance. Same trend was observed after four weeks period. SBO-based diet revealed same trend in findings of Santiago and Reyes (1993) and Nandi *et al.* (2001). Concerning linolenic acid (ALA), female broods delivered FO source in their diet in different forms ($FO_{cont.}$, $MIX_{cont.}$, FO_{am}/SBO_{pm} and SBO_{am}/FO_{pm}) for two or four weeks showed higher values of linolenic acid (C18:3 n-3) in their ovaries than those fed SBO diet continuously. Same trend was observed for other fatty acids in n-3 group where, the highest accumulation for EPA showed after two weeks of feeding broods under lipid regime of $FO_{cont.}$ and FO_{am}/SBO_{pm}. Same result was suggested for DHA, total LC-PUFA and total n-3 ($P > 0.05$) but on numerical value basis (Table 5). Four weeks results showed no significant differences for EPA, DHA, total LC-PUFA and total n-3. It is clear that female broods require a specific balanced ratio of n-6/n-3 for optimal reproductive performance. Elevated accumulation of total LC-PUFA and total n-3 may have detrimental effects on tilapia reproduction.

Table 5: Effect of lipid feeding regimes on fatty acid profile (% total fatty acids) of female ovaries after fed on the experimental diets for two weeks (means ± SE) [a].

Fatty acids	$FO_{cont.}$	$SBO_{cont.}$	$MIX_{cont.}$	FO_{am}/SBO_{pm}	SBO_{am}/FO_{pm}
C14:0	5.42±0.90	4.65±2.45	4.91±0.73	4.89±0.46	3.35±0.25
C15:0	0.78±0.10	0.64±0.23	0.63±0.09	0.68±0.05	0.51±0.06
C16:0	27.54±3.45	24.91±4.41	25.27±0.84	25.70±0.93	24.09±1.37
C17:0	0.63±0.01	0.45±0.01	0.45±0.05	0.53±0.11	0.51±0.04
C18:0	6.34±0.21	6.84±0.94	6.38±0.05	6.49±0.23	6.97±0.19
C20:0	0.60±0.23	0.67±0.20	0.74±0.09	0.66±0.02	0.77±0.02
∑ SFA [c]	41.30±4.02	38.15±5.95	38.38±1.57	38.94±1.80	36.18±1.37
C16:1	12.01±4.13	9.43±3.61	9.06±1.55	11.04±0.37	7.15±0.11
C17:1	0.48±0.03[a]	0.33±0.00[ab]	0.13±0.13[b]	ND [b]	0.38±0.09[ab]
C18:1 n-9	15.93±4.28	17.97±2.83	19.15±2.60	14.73±1.97	21.84±0.14
C20:1	0.70±0.17	1.15±0.44	0.85±0.12	0.81±0.10	0.98±0.03
C22:1 n-9	0.13±0.03	0.02±0.02	0.14±0.04	0.10±0.01	0.32±0.21
∑ MUFA [d]	29.22±0.31	28.89±0.36	29.33±1.26	26.68±2.25	30.66±0.06
C18:2 (n-6)-t	0.14±0.14	ND	0.14±0.14	0.12±0.12	0.31±0.02
C18:2 n-6	10.36±4.20	18.10±2.63	16.44±1.40	14.14±1.32	17.61±1.33
C18:3 n-3	0.28±0.03[a]	0.07±0.07[b]	0.30±0.05[a]	0.34±0.06[a]	0.31±0.06[a]
C18:3 (n-6)-γ	0.26±0.01	0.25±0.05	0.28±0.01	0.28±0.03	0.31±0.04
C20:2 n-6	0.78±0.16	0.99±0.27	0.95±0.13	0.93±0.11	0.98±0.02
C20:5 n-3	4.35±0.46[a]	2.67±0.04[b]	3.70±0.54[ab]	4.35±0.29[a]	3.59±0.25[ab]
C22:6 n-3	13.32±0.37	10.89±3.38	10.49±0.77	14.23±0.80	10.08±0.29
∑ MC-PUFA [e]	11.04±4.37	18.43±2.62	17.16±1.50	14.88±1.42	18.52±1.33
∑ LC-PUFA [f]	18.45±0.67	14.54±3.69	15.14±1.19	19.51±0.98	14.64±0.02
∑ PUFA [g]	29.49±3.70	32.96±6.31	32.30±0.31	34.39±0.43	33.16±1.31
∑ n-3 [h]	17.95±0.80	13.63±3.36	14.49±1.36	18.93±1.15	13.97±0.02
∑ n-6 [i]	11.54±4.50	19.34±2.95	17.81±1.68	15.47±1.59	19.20±1.30
n-6 : n-3	0.66±0.28[b]	1.46±0.15[a]	1.25±0.23[ab]	0.83±0.14[ab]	1.38±0.10[a]
PUFA : SFA	0.73±0.16	0.91±0.31	0.85±0.05	0.88±0.03	0.92±0.07

[a] Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.
[b] ND, not detected.
[c] Total SFA, total saturases, included 14:0, 15:0, 16:0, 17:0, 18:0, and 20:0.
[d] Total MUFA, total monoenes, included 16:1, 17:1, 18:1 n-9, 20:1and 22:1 n-9.
[e] Medium-chain PUFA, sum of all PUFA with chain length of 18 carbon atoms.
[f] Long-chain PUFA, sum of all FA with chain length ≥20 carbon atoms and ≥3 double bonds.
[g] Total PUFA included 18:2 (n-6)-t, 18:2 n-6, 18:3 n-3, 18:3(n-6)-γ, 20:2 n-6, 20:5 n-3 and 22:6 n-3.
[h] Total n-3 included 18:3 n-3, 20:5 n-3 and 22:6 n-3.
[i] Total n-6 included 18:2(n-6)-t, 18:2 n-6, 18:3(n-6)-γ and 20:2 n-6.

The harmful effects of excessive n-3 may be due to increased oxidative stress in gonad tissues as HUFA are easily oxidized create highly reactive lipid peroxidation radicals that may be detrimental to egg development or embryo (Ng and Wang, 2011). Though, more tolerance for n-3 fatty acids as more competition for the same desaturase enzymes between n-6 and n-3 groups revealed under unbalanced dietary n-6/n-3 (Al-Souti *et al.*, 2012).

Table 6: Effect of lipid feeding regimes on fatty acid profile (% total fatty acids) of female ovaries after fed on the experimental diets for four weeks (means ± SE) [a].

Fatty acids	$FO_{cont.}$	$SBO_{cont.}$	$MIX_{cont.}$	FO_{am}/SBO_{pm}	SBO_{am}/FO_{pm}
C14:0	4.12±0.78	2.19±0.26	3.30±1.08	2.65±0.25	1.43±1.17

C15:0	0.72±0.08	0.25±0.25	0.75±0.06	0.46±0.18	0.15±0.15
C16:0	31.57±3.36[a]	24.22±2.95[ab]	30.15±0.37[a]	23.44±3.89[ab]	19.06±0.54[b]
C17:0	0.58±0.17	0.48±0.01	0.80±0.10	0.44±0.10	0.58±0.25
C18:0	11.24±0.82	10.29±0.46	5.65±4.12	8.85±2.01	5.89±0.14
C20:0	1.00±0.28	0.74±0.10	0.71±0.04	0.82±0.21	1.10±0.01
C22:0	0.05±0.05[ab]	ND [b]	ND	0.12±0.12[ab]	0.23±0.00[a]
∑ SFA [c]	49.26±3.89[a]	38.18±2.91[ab]	41.36±4.72[ab]	36.76±6.09[ab]	28.42±0.87[b]
C16:1	10.80±2.07[a]	5.01±0.96[b]	4.98±0.52[b]	9.37±0.64[a]	11.04±0.35[a]
C17:1	0.19±0.19	0.20±0.02	0.17±0.17	0.35±0.00	0.19±0.19
C18:1 n-9	16.42±4.23	18.80±0.87	12.03±1.48	14.76±7.39	22.50±0.86
C20:1	0.64±0.04[c]	1.59±0.10[a]	1.15±0.01[b]	1.16±0.14[b]	1.04±0.02[b]
C22:1 n-9	0.08±0.08	ND	ND	ND	ND
∑ MUFA [d]	28.11±6.22	25.60±0.01	18.31±1.14	25.64±8.17	34.76±1.38
C18:2 n-6	6.88±2.03[b]	19.62±2.00[a]	14.75±1.59[ab]	12.53±3.82[ab]	16.13±0.44[a]
C18:3 n-3	0.31±0.01[a]	ND	0.19±0.00[b]	0.01±0.01[c]	0.20±0.01[b]
C18:3 (n-6)-γ	0.37±0.07	0.32±0.02	0.38±0.05	0.47±0.13	0.24±0.03
C20:2 n-6	0.62±0.13[b]	1.19±0.17[a]	0.92±0.05[ab]	0.96±0.16[ab]	0.78±0.12[ab]
C20:5 n-3	3.20±1.63	2.14±0.26	4.05±1.07	4.61±1.13	3.34±0.12
C22:6 n-3	11.27±6.38	12.98±0.99	20.06±6.29	19.04±4.47	15.07±0.94
∑ MC-PUFA [e]	7.55±1.97[b]	19.93±2.01[a]	15.31±1.54[ab]	13.01±3.69[ab]	16.56±0.47[a]
∑ LC-PUFA [f]	15.08±8.14	16.30±0.90	25.03±7.40	24.61±5.76	19.18±0.70
∑ PUFA [g]	22.63±10.11	36.23±2.92	40.34±5.86	37.61±2.07	35.74±1.17
∑ n-3 [h]	14.78±8.02	15.11±0.74	24.30±7.35	23.66±5.61	18.60±0.84
∑ n-6 [i]	7.86±2.10[b]	21.12±2.18[a]	16.04±1.50[ab]	13.96±3.54[ab]	17.14±0.34[a]
n-6 : n-3	0.65±0.21	1.40±0.08	0.75±0.29	0.67±0.31	0.93±0.03
PUFA : SFA	0.48±0.24[b]	0.96±0.15[ab]	1.01±0.26[ab]	1.05±0.12[ab]	1.26±0.08[a]

[a] Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.
[b] ND, not detected.
[c] Total SFA, total saturases, included 14:0, 15:0, 16:0, 17:0, 18:0, 20:0 and 22:0.
[d] Total MUFA, total monoenes, included 16:1, 17:1, 18:1 n-9, 20:1and 22:1 n-9.
[e] Medium-chain PUFA, sum of all PUFA with chain length of 18 carbon atoms.
[f] Long-chain PUFA, sum of all FA with chain length ≥20 carbon atoms and ≥3 double bonds.
[g] Total PUFA included 18:2 n-6, 18:3 n-3, 18:3(n-6)-γ, 20:2 n-6, 20:5 n-3 and 22:6 n-3.
[h] Total n-3 included 18:3 n-3, 20:5 n-3 and 22:6 n-3.
[i] Total n-6 included 18:2(n-6)-t, 18:2 n-6, 18:3(n-6)-γ and 20:2 n-6.

Ratios of n-6/n-3 in broodstock ovaries for fish fed $SBO_{cont.}$, $MIX_{cont.}$ and SBO_{am}/FO_{pm} regimes reached 1.46, 1.25 and 1.38, respectively after two week of conditioning period. These ratios were consistent with the recommended ratios for Nile tilapia broodstock (1.4 and 1.9, respectively) as suggested by Santiago and Reyes (1993) and Ng and Wang (2011). With more accumulation of n-3 fatty acids under condition of sex separation and no spawning process, broods fed under the last feeding regimes for four weeks showed a decrease in ovaries ratio of n-6/n-3 to become 1.40, 0.75 and 0.93, respectively. The last ratios were slightly lower than those recommended for Nile tilapia broods. Based on these results, conditioning of broodstock for two weeks period is recommended than four weeks.

Females fed same lipid sources and ratios of FO and SBO (1:1) but in different time of the day showed different ovaries fatty acid profiles. Feeding females under lipid regime of FO_{am}/SBO_{pm} for two or four weeks accumulated EPA, DHA, total LC-PUFA and total n-3 more than those fed the same lipid sources under SBO_{am}/FO_{pm} regime. These results revealed that fish might prefer to accumulate lipid sources in the morning period, while consuming them in the afternoon period as energy source for different activities. Reproductive performance previously presented confirmed the same hypothesis. Delivering SBO in the morning and FO in the afternoon led to better fatty acid profile for tilapia broods, subsequently better reproductive performance than FO_{am}/SBO_{pm} regime. The physiological effect of day time on lipid utilization may be suitable for application in washing out strategy experiments or grow out phase of marine fish species. Delivering high expensive

lipid source such as fish oil in early morning diet is more suitable to avoid losing it as energy source.

Gonado-somatic index (GSI):

The effect of lipid feeding regimes on GSI after either two or four weeks of conditioning periods is tabulated in Table7. No significant differences were recorded among different lipid feeding regimes regarding GSI even after two or four weeks. Fish fed SBO_{am}/FO_{pm} diet had numerically higher GSI value than other treatments after 2wks subsequently after 4wks (Table 7). Santiago and Reyes (1993) who found that GSI of tilapia brood females did not significantly different among fish fed fish oil, soybean oil, coconut oil or corn oil diets. Ng and Wang (2011) found no significant differences regarding GSI values among tilapia broodstock fed vegetable oil based-diets (crude palm oil or crude palm oil + fish oil) and those fed fish oil diet. However, females fed vegetable oil based-diets had numerically higher GSI than those fed fish oil diet.

Table 7: Gonado-somatic index of females fed different lipid regimes for two weeks and four weeks (means ± SE) [a].

	Effect of lipid feeding regimes				
	$FO_{cont.}$	$SBO_{cont.}$	$MIX_{cont.}$	FO_{am}/SBO_{pm}	SBO_{am}/FO_{pm}
Initial	4.47	4.47	4.47	4.47	4.47
2Wks.	4.15±2.25	4.93±0.82	4.79±0.15	4.38±1.64	6.75±0.24
4Wks.	2.81±1.40	3.16±0.34	1.94±0.98	2.87±0.88	3.87±0.59
	Effect of periods				
	Initial		*After two weeks*		*After four weeks*
	4.47±0.41[a]		5.00±0.41[a]		2.93±0.41[b]

[a] Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.

Irrespective of lipid feeding regimes, a significant reduction in GSI was noticed after four weeks in comparison with initial and two weeks of conditional period. The highest significant value was recorded for tilapia broodstock after two weeks period (Table 7). The reduction in ovary size may be due re-absorption of well-developed egg in the ovary after long conditioning period, same was suggested by Peters (1983) and Little *et al.* (1993). The importance of conditioning period for tilapia broodstock was reported by many authors with different suggestions for the optimal conditioning period. Short resting period (5-15 days) with good feeding regimes for Nile tilapia broodstock was suggested for its improvement effect on the reproductive performance in comparison with non rested broodstock (Little *et al*., 1993; Little *et al*., 2000; Bhujel, 2000, El-Sayed, 2006; Abou-Zied, 2015). In the same context, Costa-Pierce and Hadikusumah (1995) stated that tilapia broodstock conditioned for 14-16 days had the highest fry production than broodstock conditioned for 21 or 30 days. In contrast, other authors suggested that long resting period had better effect on reproductive performance than no resting period, Lovshin and Ibrahim (1988) found that conditioning period of 21 days for both male and female increased the reproductive performance by 16%.

Reproductive performance of Tilapia broodstock:

The reproductive performance was followed after 4wks of conditioning period for tilapia broodstock and two batches were recorded in a period of 14 days and after 25 days. Fish fed SBO-based diets in forms $SBO_{cont.}$, $MIX_{cont.}$ or SBO_{am}/FO_{pm} generally showed the best reproductive performance. The highest significant seed production, absolute fecundity and system productivity were recorded for fish fed $MIX_{cont.}$ and SBO_{am}/FO_{pm} diets followed by fish fed $SBO_{cont.}$ diet without any

significant differences. The lowest values were recorded for fish fed $FO_{cont.}$ or FO_{am}/SBO_{pm} diets. Fish fed $SBO_{cont.}$, $MIX_{cont.}$ and SBO_{am}/FO_{pm} showed slightly better relative fecundity but not significantly than those fed $FO_{cont.}$ or FO_{am}/SBO_{pm} diets (Table 8).

Table 8: Effect of lipid feeding regimes on reproductive performance of tilapia females broodstock (means ± SE) [a].

	Treatments				
	$FO_{cont.}$	$SBO_{cont.}$	$MIX_{cont.}$	FO_{am}/SBO_{pm}	SBO_{am}/FO_{pm}
Total seed production	1171±179[b]	1930±308[ab]	2293±248[a]	1250±205[b]	2278±376[a]
Absolute fecundity [b]	293±45[b]	483±77[ab]	573±62[a]	313±51[b]	569±94[a]
Relative fecundity [c]	3.23±0.58	5.12±0.85	5.83±0.78	3.47±0.53	6.21±1.07
System productivity [d]	26.09±3.99[b]	43.00±6.87[ab]	51.08±5.52[a]	27.85±4.57[b]	50.74±8.37[a]

[a] Means in the same row with different superscripts are significantly different ($P \leq 0.05$) by Duncan's test.
[b] Absolute fecundity = Mean number of seeds at each spawning per female.
[c] Relative fecundity = Mean number of seeds at each spawning per female body weight (g).
[d] System productivity =Mean number of seeds per day/ hapa size.

Many authors suggested that vegetable oils or mixture of fish oil and vegetable oil resulted in better reproductive performance than fish oil solely. Santiago and Reyes (1993) reported that fish fed SBO diet had the best reproductive performance, while fish fed cod liver oil diet showed the poorest reproductive parameters. Ng and Wang (2011) found that tilapia broodstock fed crude palm oil-based diets (CPO or FO+CPO 1:1) showed the highest total eggs, higher spawning frequency and the best egg hatchability than broodstock fed FO or LSO diets this result may be due to the shorter inter spawning interval and higher spawning frequency. Tilapia broods fed mixture of palm oil+fish oil(9:1) diets showed better reproductive performance than those fed palm oil-based diet (Hajizadeh *et al.,* 2008). Furthermore, Nandi *et al.* (2001) observed that the broodstock females carp *Catla catla* fed mixture diet (SBO+FO 9:1) had the highest fully matured females and relative fecundity followed by fish fed SBO diet without significant differences, while broods fed FO diet showed the lowest reproductive performance. The same were confirmed by the results reported in Table 8, where tilapia broodstock that fed MIX diets in forms ($MIX_{cont.}$ or SBO_{am}/FO_{pm}) showed generally the best reproductive performance followed by fish fed SBO diet without any significant differences. Based on these results it could be suggested that despite the importance of n-6 fatty acid family for tropical fish like tilapia (Teshima *et al*., 1982; Lovell, 1989; NRC, 1993; Popma and Masser, 1999; Shiau, 2002), it seems that broodstock may also need lesser quantities of n-3 fatty acid family (Santiago and Reyes, 1993; Nandi *et al.,* 2001; Hajizadeh *et al.,* 2008) to obtain the best reproductive performance. Otherwise, El-Sayed *et al.* (2005) stated that no significant differences for reproductive performance among fish fed FO, SBO or MIX diets for 165 days under condition of 0% salinity.

Despite fish have delivered mixture of FO and SBO (1:1) in different feeding regimes ($MIX_{cont.}$, SBO_{am}/FO_{pm} or FO_{am}/SBO_{pm}) but only two of these feeding regimes ($MIX_{cont.}$ and SBO_{am}/FO_{pm}) showed the highest reproductive performance which support our hypothesis that tilapia ability to utilize lipid sources depend widely on time of the day. It was recognized from the obtained results that tilapia utilizes lipid sources in the morning better than afternoon. However, it was observed that Nile

tilapia utilizes food more efficiently during afternoon period (El-Husseiny *et al.*, 2004). As enzyme activities may be responsible for different nutrient utilization in afternoon period (Hardland *et al*., 1973). In the same context, Suloma *et al.* (2017) found that tilapia showed better utilization for dietary protein in the afternoon than in the morning period. Regarding fry quality, broodstock fed SBO continuously and SBO_{am}/FO_{pm} diets produced numerically higher fry weight than other treatments (Table 9). This result is in parallel with results of reproductive performance.

Table 9: Fry weight produced from broodstock fed different lipid regimes.

	Treatments				
Fry weight (mg)	$FO_{cont.}$	$SBO_{cont.}$	$MIX_{cont.}$	FO_{am}/SBO_{pm}	SBO_{am}/FO_{pm}
First batch	15.22	20.11	14.73	14.95	20.67
Second batch	15.60	24.90	15.20	18.60	16.90
Average	15.41	22.50	14.96	16.78	18.79

In conclusion, under condition of hapa-in-pond system, the optimal n-6/n-3 ratio could be reached after conditioning period of two weeks. More elongation may drop the ratio to inappropriate limits. Mixture of FO and SBO resulted in the best reproductive performance than sole oil based-diet. Preferred consumption of the lipid as energy source for different activity was noticed in the afternoon period, while more accumulation trend occurred in the morning period. Delivering same lipid sources in different time of the day led to different fatty acid profiles. Further work may be needed to examine the effect of lipid feeding regime on other field of studies as washing out strategies or grow out phase of marine fish species.

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ARABIC SUMMARY

تأثيرأنظمة تغذية الدهون وفترات الإعدادعلى محتوى الغدد التناسلية من الأحماض الدهنية و الأداء التناسلي لأمهات البلطي النيلي (*Oreochromis niloticus*)

أسامة محمد الحسيني[١] , أشرف محمد عبد السميع جودة[٢] , رانيه سعيد مبروك[١] , علي مطر[٢]

١- قسم الإنتاج الحيواني، معمل تغذية الأسماك (FNL) ، كلية الزراعة، جامعةالقاهرة، ١٢٦١٣ القاهرة، مصر

٢- معمل تغذية الأسماك، المعهدالقومي لعلوم البحار والمصايد، القاهرة، مصر

في ظل نظام تربية الأحياء المائية في هابات ، تم دراسة عدة نظم تغذية على مصادر مختلفة من الدهون وفترات زمنية مختلفة لإعداد أمهات البلطي النيلي ودراسة تأثيرهاعلى محتوى مبايض الأمهات من الأحماض الدهنية وعلى أدائهم التناسلي. حيث تم تكوين عليقة أساسية تختلف في مصدر الدهون لتكون ثلاثة علائق أ- عليقة أساسية + زيتالصويا (صويا), ب- عليقة أساسية + زيت السمك, ج- عليقةأساسية + خليط من زيت السمك : زيت الصويا ١:١ (خليط). تم استخدام هذه العلائق الثلاثة لتنفيذ خمسة أنظمة غذائية. ١- عليقة زيت السمك تقدم بشكل مستمر خلال فترة التجربة (سمك مستمر), ٢- عليقة زيت الصويا بشكل مستمر خلال فترة التجربة (صويا مستمر), ٣- عليقة زيت السمك في الصباح ثم زيت صويا في فترة مابعد الظهر (سمك ص / صويا م) , ٤- عليقة زيت الصويا في الصباح ثم زيت السمك في فترة ما بعد الظهر (صويا ص / سمك م) ,٥- عليقة خليط من زيت السمك : زيت الصويا ١:١بشكل مستمر خلال فترة التجربة (خليط مستمر). تم فحص المعاملات تحت فترة اعداد للامهات لمدة أسبوعين وأربعة أسابيع. وجد أن مبايض الإناث التي تغذت على علائق قائمة على الصويا سواء: (صويا مستمر) , (خليط مستمر) أو (صويا ص / سمك م) احتوت على قيم أعلى من إجمالي n-6 ونسبة n-3 / n-6 وأفضل أداء تناسلي. ولوحظ الاتجاه المعاكس بالنسبة للإناث المتغذية على علائق (سمك مستمر) و (سمك ص / صويا م). و لوحظ أن نسبة n-3 / n-6 للأسماك التي تغذت على علائق (صويا مستمر) , (خليط مستمر) أو (صويا ص / سمك م) وصلت للنسبة الموصى بها كافضل أداء تناسلي بعد أسبوعين من فترة الإعداد. ايضا وجد أن المبايض تخزن الأحماض الدهنية في فترة الصباح أكثر من فترة بعد الظهر. وأوضحت النتائج أن الأداء التناسلي المثالي لأمهات البلطي النيلي يحتاج الى زيت السمك للوصول بنسبة n-3 / n-6 الى النسبة المثالية في فترة قصيرة وقد يؤدي تقديم زيت السمك في الصباح إلى ضمان الاستفادة القصوى من هذا المصدر المكلف.