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HORMONAL INDUCTION AND SYNCHRONIZATION IN THE REPRODUCTION OF NILE TILAPIA (*OREOCHROMIS NILOTICUS*)

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Abstract

The present study aims to evaluate the sexual synchronization of Nile tilapia (Oreochromisniloticus) breeding herds under different distributions and days. This study was carried out during, a period of 21 experimental days. It was conducted in a randomized block design (DBC), in a factorial scheme 2 × 2 × 3, (male and female, with and without hormone induction, and three times blocks of 7, 14 and 21 days). A total of 324 fish were used, 180 females and 144 males, previously microchipped with approximately 250 ± 12.25 g of body weight.. Each tank contained 36 animals, with every three tanks comprising a block of which were repeated in time. Each of the tanks was composed of only males, another with 12 males and 24 females separated by a glass and a screen on the bottom and the third tank with only females. In each treatment, half of the animals (male and female) were applied the hCG hormone, in a single dose at a concentration of 5 IU / gram fish live weight. The animals were evaluated at 7, 14, and 21 days. After 667 hours-degree after application of the hormone was made extrusion and spermatozoa by celomatic massage. It was observed females that showed ease of extrusion of gametes. For the females, spawning index, absolute and relative fecundity, oocyte weight per 1 g, diameters, and germinal vesicle peripheral position were evaluated; the sperm concentration, volume (ml), motility (%), duration of sperm motility (min) and integrity were evaluated in males. The data were submitted to analysis of variance at the 5% level of significance. For females at 14 days there was greater ease of extrusion when separated from males, with hormone application . The distribution influenced the spawning weight, the larger diameter, and for the diameters smaller the days and induction presented interference. In males, the days interfered in the increase in volume and duration of semen motility independent of induction and environment, and finally, the environment interfered significantly in semen concentration.

Keyword

hCG. Reproduction. Reproductive performance.

SINCRONIZAÇÃO E INDUÇÃO HORMONAL NA REPRODUÇÃO DE TILÁPIA DO NILO *(OREOCHROMIS NILOTICUS)*

Resumo

O presente estudo tem como objetivo avaliar a sincronização sexual de reprodutores de tilápias do Nilo (Oreochromis niloticus) em cativeiro sob diferentes distribuições e dias. Este estudo foi realizado por um período de 21 dias experimentais. Foi conduzido num delineamento de blocos casualizados (DBC), num esquema fatorial 2×2×3, (sendo macho e fêmea, com e sem indução hormonal) e três tempos, 7, 14 e 21 dias (blocos). Foram utilizados 324 peixes, sendo 180 fêmeas e 144 machos, com aproximadamente 250 ± 12,25g de peso corporal, previamente microchipados. Cada tanque continha 36 animais, sendo que a cada três tanques compreendia um bloco do qual foram repetidos no tempo. Cada um dos tanques esteve composto por: somente machos, outro com 12 machos e 24 fêmeas separados por um vidro e uma tela na parte de baixo e o terceiro tanque com apenas fêmeas. Em cada tanque, metade dos peixes (macho e fêmeas) recebeu indução hormonal com hCG (CHORULON®), na concentração de 5000UI/grama de peso vivo por peixe. Os animais foram avaliados aos 7, 14, e 21 dias. Passado 667 horas-grau após aplicação do hormônio foi feita extrusão e espermeação mediante massagem celomática. Foram observadas fêmeas que apresentaram facilidade de extrusão de gametas. Para as fêmeas foram avaliadas as seguintes variáveis: índice de desova, fecundidade absoluta e relativa, o peso dos ovócitos por cada 1g, os diâmetros, e posição periférica da vesícula germinativa; nos machos, foram avaliados a concentração espermática, o volume (ml), motilidade (%), tempo de duração de motilidade espermática (min) e integridade. Os dados obtidos foram submetidos a análise de variância ao nível de 5% de significância. Observou-se para fêmeas aos 14 dias, mantidas separada dos machos e com aplicação de hormônios, maior facilidade de extrusão, peso da desova diâmetro dos ovócitos. Nos machos, os dias interferiram no aumento do volume e na duração de motilidade do sêmen independente da indução e ambiente, e por fim, o ambiente interferiu significativamente na concentração do sêmen.

Palavra-chave

Desempenho reprodutivo. hCG. Reprodução.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is the second most farmed group of fish in the world. According to the United Nations Organization for Food and Agriculture forecast, (2018), tilapia production will increase in 30% until 2030. In Brazil, Nile tilapia is the most farmed specie in fish farming with annual production of 432.149 tonnes representing 57% of the total production (PEIXE BR, 2020).

The asynchronous spawning characteristic for genetic improvement becomes a hindrance for identification of the families and in accurate prediction of their genetic value, thus the synchronization production in tilapia with manipulation of hormone inductors and the matrix may bring needed information to obtain homogenous families, *in vitro* fertilization and in parental identification. Hormonal induction may also be used to anticipate the production period, to restrict it or even to synchronize the production of a matrix lot. These factors enables the producer to obtain juvenile fish in periods when the profit is higher, or to finish farming at time when commercialisation is optimized. (PAULINO et al., SOUZA et al., ANDRADE et al., 2016).

External periodic variations are used by animals to adjust their biologic rhythms called synchronizers, which can be classified in biotic and abiotic. Biotic factors are the main daily behaviour rhythm synchronizers of the gene expression (DIAS e MARIANO, 2015) and it has a critical role in seasonal rhythm, as the fish production (NAVARRO e NAVARRO, 2012).

Reproductive behaviour is also influenced by endogenous factors, which start and control hormonal alterations, gonadal and morphologic, thus determining when the isogenic factors will be functioning (ANDRADE et al, 2016). And the exogenous factors are factors that prepare the animal so that the reproduction takes place in environmental adequate conditions (REBOUÇAS. 2014; PAULINO et al., 2016 e NUNES et al., 2018).

These exogenous factors enable the big fish fry producers to obtain fish fry in periods of high profitability, or when the cultivation can be finalized in periods in which commercialization can be optimized (ANDRADE et al e PAULINO et al., 2016).

The use of hormonal technique administration in fish enable programming

the production, facilitating the methods of genetic family evaluation and in accurate prediction of their genetic values, which was the complicator when there was no knowledge of reproductive control technique in the specie SOUZA et al., 2016 and NUNES et al., 2018.

The objective of the present study is to evaluate the influence of male/female relationship in hormonal induction to stimulate the synchronization in production of Nile tilapia (*Oreochromis niloticus*) as an important tool in genetic improvement of the specie.

MATERIAL AND METHODS

The adopted approaches during the experiment were approved by the Universidade Federal de Lavras commit of Ethics for Animal Use, protocol n° 056/18.

The study was conducted in the sector of Pscicultura do Departamento de Zootecnia da Universidade Federal de Lavras, Lavras-MG, between March and April, totalizing 21 days of experiments. Nile tilapia specimens were obtained from the animal herd of the same sector. There were used 324 fish, 180 female and 144 male, previously microchipped with an average weight of approximately 250±12,25g of mass.

The animals were distributed in 9 masonry tanks, with the following dimensions 2X4x1,5 (width/length/depth) and continuous flow of water. Daily the fish were fed *ad libitum* at 8h and at 14h with an extruded commercial diet containing 36% of protein. Then, were carried out average measurements of, temperature (20±3,79 °C) and pH (7,8±0,3 for whole experimental period.

The female were selected, through observation of the secondary characteristics of sexual maturation quoted by (FELIZARDO et al., 2010 and SOUZA et al., 2016). The males in turn, were selected through reproductive characteristics quoted by PAULINO et al., 2016 e SOUZA et al., 2018).

Every 3 (three) tanks of the experiment, composed 1 (um) experimental block, repeated in time. Each tank received 36 animals, distributed in the following form: (T1) 36 males, (T2) 12 males e 24 females separated by a glass and a screen at the bottom and (T3) with 36 females.

The animals were distributed in three tanks and evaluated during 21 days of which, each period was of 7, 14, 21 experimental days.

The criteria for use of size of sample in tanks which contained males and females was based on previous studies (NASCIMENTO et al., 2014 e CAMPOS et al., 2018 ;). Thus, the present study follows established proportions, taking also into account the size of the masonry tanks available for the study, with the density that neither compromise the growth of the animals nor provoke stress by overpopulation.

For the tanks which contained only males or only females, the criteria used was the number of fish pre- established in treatment together with male and female, so that the density was not the source of variation of treatment.

After 21 experimental days, taking into account they had completed 14 and 7 days, all were submitted to a period of food restriction of 24 hours. After this food restriction, half of the animals of each tank were artificially inducted with a unique dose, intramuscular injection, at the base of the dorsal fin with Human Chorionic Gonadotropin (hCG*), CHORULON® 5000 UI* per gram weight of a live fish (SOUZA et al., 2018) and the other half received saline olution.

After the hormonal induction, the tanks temperature was measured every hour in order to obtain the moment of 667 hour-degree of extrusion, and when it was obtained this time was performed the gamete extrusion (SEVIGNANI et al., 2020). All the females and males belonging to all treatments and times, submitted to hormonal induction or saline solution, individually, had the urogenital papilla and surroundings surfaces cleaned and dried with a tissue and then submitted to abdominal massage with a smooth anteropostrior movement for the total extrusion of the gamete. During the extrusion of the female oocytes, the degree of difficult for obtaining of the oocytes through abdominal massage of female were, of which were classified as easy or difficult. The female classified as easy were those without much pressure in abdominal cavity they already released the oocytes and to be classified as difficult it was necessary to be performed several smooth repeated massage for releasing.

The collected oocytes were weighed and 1g was removed in order to determine the number of oocytes /g. The diameter (mm) was measured in 10 of oocytes every female, previously immersed in Gilson solution (5mL of alcohol 60%, 44 mL of distilled water, 0,7g of nitric acid 80%, 1g of mercuric chloride e 0,9mL of glacial acetic acid).

For the analysis of peripheral position of germinal vesicle (PPVG %), 10 oocytes of each female were immersed in an ethanol solution, formaldehyde and

sulfuric acid (60:39:1). The analysis of diameter and PPVG was carried out with the help an optical microscope (Nikon Eclipse E200) on the lens of 40x (Vazzoler, 1996).

After verifying the quantity of oocyte/g of each fish, the following calculations were performed: spawning index ID= (PD/PF) x100 where: PD spawning weight and PF= weight of the female subtracting the PD; absolute fecundity (FA=PD x number of oocyte in 1g), which results in total number of oocytes produced by each fish; absolute relative fecundity for the weight (FARP=(FA/PT)), being the total number of oocytes per gram of the sample, where PT= total weight of the animal, and absolute relative fecundity for the length (FARC=FA/CT) equivalent to the number of oocytes per centimeter of animal, where CT= total length of the animal

The semen of each male was collected through abdominal compression and removed with the help of a syringe of 3 ml. The following characteristics of semen were evaluated: total volume (ml), concentration (number of spermatozoa/mL), motility rate (%) duration of motility (ies) and integrity.

For the evaluation of spermatic concentration of semen *in natura* a dilution in formaldehyde solution was made: citrate in proportion of 10:1000 *(*semen: solution). The evaluation was realized in chamber of *Neubauer* in optical microscope (Nikon Eclipse E200).

The spermatic concentration was estimated by the formula: *(spermatozoa concentration/mL) = sum 5squared x104 (depth, diameter and Neubauer correction factor) x dilution.*

The rate of motility and duration of spermatic motility were verified in an aliquot of 10µL of semen in a histological slide previously activated with water in proportion of 1:5 (semen: water) viewed under optic microscope (Nikon Eclipse E200) in a 400x zoom. The motility rate was evaluated through average percentage of mobile spermatozoid in three random fields. The duration of motility, in minutes, was recorded since the activation until only 10% of the spermatozoa were active (FELIZARDO et al., 2010 PAULINO et al., 2018).

The integrity was evaluated through an aliquot of 10µL of semen in a histological slide where added necrosin and eosin colouring for subsequent scrub was and checked in an optical microscope (*Nikon Eclipse E200*) for identification of viable and unviable cells.

The reproductive parameters were evaluated by methodologies used by

Pereira et al. (2016) e Souza et al. (2018).

The design used was a randomized block design (DBC), in a factorial scheme of 2×2×3, (relationship male/female, hormonal induction (inducted or not), and time of evaluation (7, 14 and 21 day)).

The obtained data was submitted to variance analysis adopting the level of 6% of significance through Computational R Program.

RESULTS

The results found for the answers of the animal to induction are presented on table 1. The tilapia responded to hormonal induction as a regulator of productive behaviour, depending on the days and the environment where they are, providing guarantees in extrusion facility of gametes, for possible manipulation and fertilization in gamete selection programs.

Table 1- descriptive analysis of percentage (%) of female tilapias which represent facility or difficult of extrusion when kept in different distributions and experimental days.

Days	N	Distribution	Easy (%)	Difficcult (%)
7	n=24	jun-sor	66,67	33,33
	n=36	jun-hor	50,00	50,00
		sep-sor	50,00	50,00
		sep-hor	55,56	44,44
14	n=24	jun-sor	66,67	33,33
	n=36	jun-hor	75,00	25,00
		sep-sor	24,41	50,79
		sep-hor	83,33	16,67
21	n=22	jun-sor	66,7	27,27
	n=36	jun-hor	81,82	18,18
		sep-sor	72,22	27,78
		sep-hor	72,22	27,78

Legend: jun-sor = females together the males without hormonal induction; jun hor= female together with the males with hormonal induction; sep-soro = females separated from males without hormonal induction; sep-hor = females separated from males with hormonal induction.

It is possible to note that the animals with hormonal induction at 7 days of placement showed greater ease of extrusion when kept together with male with visual and chemical contact, and when placed together the males with induction hCG, and separated from males without the inductor they were difficult to extrude. However, in this period, at least 66, 67% of the females were considered of ease extrusion.

Inducted females after 14 days of placement showed greater facility extrusion

when separated from males with administration of the inductor hCG, and considered difficult to extrude when they were placed separated without administration of hormones. In this period, 83, 33% of the females which were separated with hormonal induction were considered of ease extrusion. In this same period, the hormonal induction provided an increase of 16, 66% in the females which were separated.

The females studied for 21 days, regarding extrusion, presented greater facility, when they were together with the male maintaining visual and chemical contact, with administration of hormone, and difficult when they were separated independent of induction. In this period, at least 81, 82% of the females were considered of ease extrusion together with hormonal induction.

It is possible to observe greater percentage of females of ease extrusion at 14 days and at 21 days with hormonal induction regardless of the distribution and days they presented better results for facility.

In the condition in which the animals were evaluated, there was significant ($p<0, 05$) only for the variable weight of oocytes, small diameter of oocytes spermatic volume, spermatic concentration an duration of spermatic motility.

The environment influenced the weight of spawning of the female kept with males independent of hormonal induction and the evaluation time because they presented an increase ($P<0,06$) in the total weight of spawning, (Figure 1).

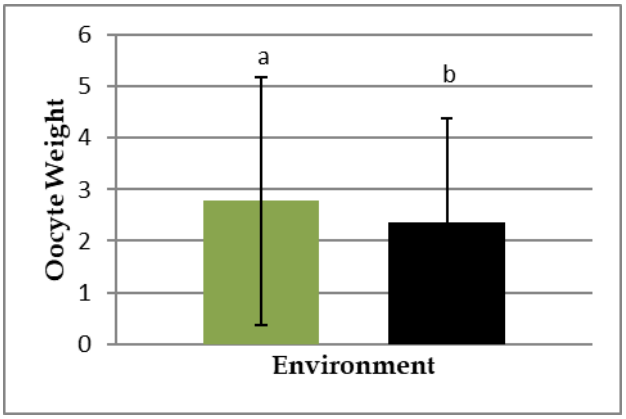

Figure 1. Average weight (g) of oocyte of tilapia (*O. niloticus*) submitted to different distributions. [a,b] Averages followed by different letters show differences between them.

The time of evaluation influenced the smaller diameter ($P <0, 06$), being that at 14 days and at 21 days there was a greater number of females with an increase of smaller diameter regarding the 7 days. The hormone induction did not influence this variable.

For the diameter evaluation of the bigger oocyte (mm), there was no influence of any of the factors evaluated (P >0, 06) (Figura 2).

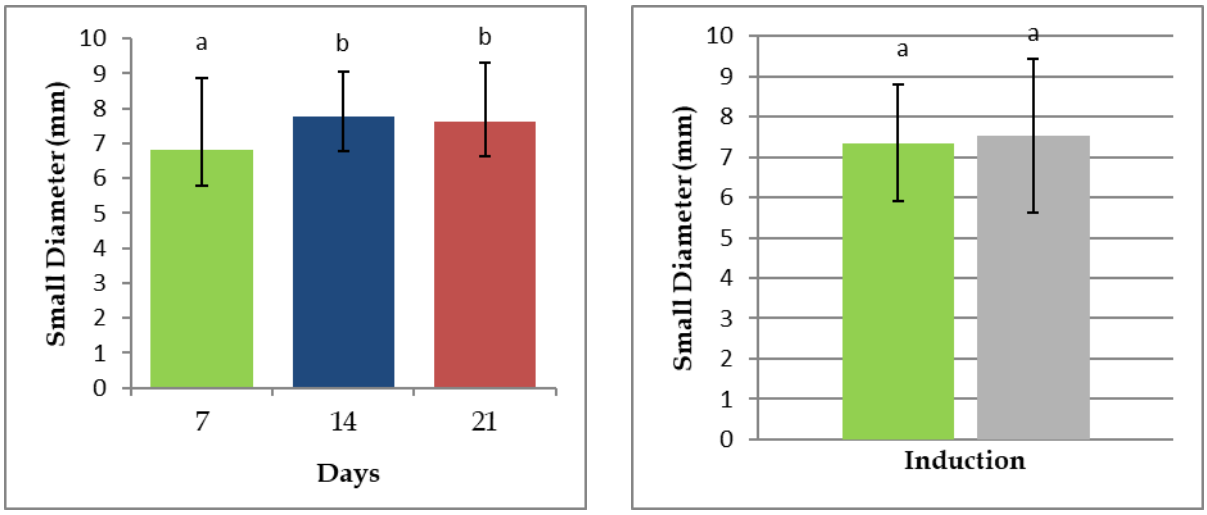

Figure.2 Smaller diameter (mm) of oocytes of tilapia (*O. niloticus*) submitted to different distributions. [a,b] Average followed by the same letter do not differ between them by the Tukey test.

For the quantity of oocyte and the position of the germinal vesicula there was no significant differences (P> 0,06) in the studied conditions, since the absolute fecundity average observed on the days was homogeneous, is was obtained 377,72 at 7 days, 452,28 at 14 days, 379,56 at 21 days. Absolute fecundity obtained higher average at 14 days, but insufficient to demonstrate statistical differences regarding hormonal induction. The placement days and the distributions (presence or absence of male) and the hormonal induction did not influence these variables.

For the spawning index, the distributions, the days and the induction, did not show significant differences (P> 0, 05).

With regard to weight of spawning it was possible to verify that the females did not present an increase of spawning weight by administration of hormone, distributions and days of placement. (Table 2).

The results found for the seminal volume in relationship to days of distribution are presented in Figure 3. Independent of the induction and the relation male and female it is possible to verify that the days influenced in the increase of semen volume (P <0, 06) at 14 days.

In this study, the level spermatic concentration were higher in males separated from females independent of administration of hormones and of the relationship male and females (P <0,06) (Figure 4).

Table 2. Averages and reproductive parameter deviations of female tilapia at 7 days, at 14 days and at 21 days of the experiment under different distributions and induction.

	Variables	P.D (g)	I.D%	F.A	Q.O (1g)	FARP	FARC	PPVG (%)
Traments 7 Days	Jun+ sor	3,73±3,02	1,32±1,24	506,10±432,64	120,6±36,30	2,11±1,94	21,38±17,46	3,78±1,98
	Jun +hor	2,11±1,76	0,90±0,51	395,08±321,31	141,9±72,65	1,47±0,96	16,02±12,15	4,00±1,09
	sep+sor	1,97±1,78	0,92±0,69	300,18±222,88	120,0±88,20	1,27±0,94	12,55±9,20	2,94±1,91
	sep+hor	2,09±1,54	1,11±0,77	309,52±227,23	152,26±76,88	1,62±1,07	13,98±10,03	3,88±1,40
Traments 14 Days	jun+sor	2,43±2,11	1,06±0,99	405,48±383,34	111,66±87,70	1,71±1,59	18,07±16,81	5,00±0,81
	jun+hor	2,93±3,04	1,65±1,26	536,93±469,95	88,75±65,88	2,22±1,88	23,48±20,03	4,42±2,29
	Sep+sor	1,09±1,28	0,53±0,53	199,10±225,45	116,17±84,92	0,80±0,87	8,51±9,14	6,46±1,76
	Sep+hor	4,40±3,70	1,51±1,20	667,61±629,06	160,37±57,44	2,24±1,87	26,60±23,31	4,47±0,94
Traments 21 Days	jun+ or	2,46±2,14	1,15±1,13	449,99±363,86	182,81±132,64	2,02±1,73	20,59±16,91	3,66±1,00
	jun +hor	3,00±2,37	1,32±1,30	543,07±298,13	213,72±149,29	2,15±1,60	23,91±13,62	4,90±1,81
	sep+sor	1,77±0,87	1,75±1,58	564.09±94,34	224,39±97,58	3,72±1,07	16,04±11,87	4,12±1,25
	sep+hor	2,78±2,95	1,12±1,11	448,69±476,18	150,88±77,63	1,86±1,91	20,83±22,48	4,12±1,54

P. Spawning weight, I. Spawning Índex, F. Absolute Fecundity, Q. oocyte = Oocyte quantity in each 1g, FARC= Absolute Fecundity relative to weight; FARC= Absolute Fecundity relative to length; PPVG= Peripheral Position of Germinal Vesicula. jun-sor = females together with males without hormonal induction; jun-hor = females together with males with hormonal induction; sep-soro = females separated from males without hormonal induction; sep-hor = females separated from males with hormonal induction.

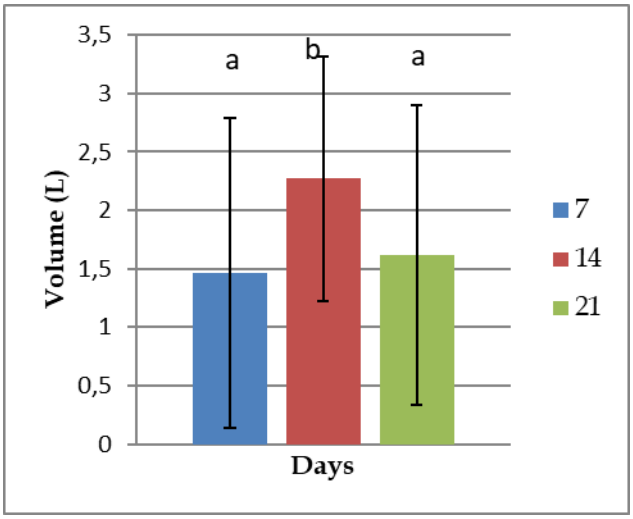

Figure 3. Average Volume (mL) of tilapia semen (*O niloticus*) submitted to treatment up to 21 days. [a,b] Averages following by the same letter do not differ between them by the Tukey test.

For the duration of motility, in this study it was verified that the days also influenced in the results being higher at 14 days (P <0,06), (Figure 5).

For the motility variables it was not found statistics differences between the treatments (P> 0,06), presenting average rates of 87,77 % at 7 days , 98,33% at 14 days and 78% at 21 days table 2. For the analysis of seminal integrity the treatment did not show significant differences.

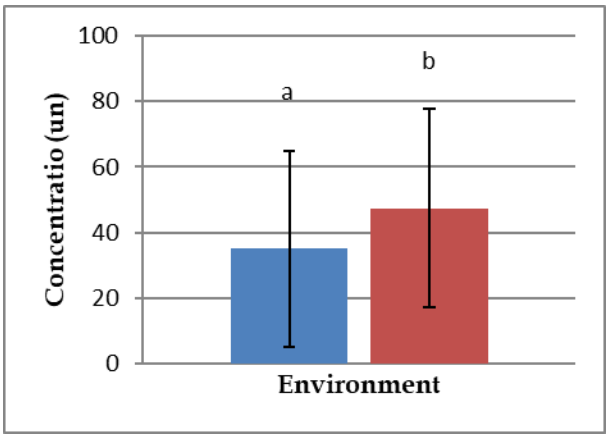

Figure 4. Concentration average (×10⁹ mL) of spermatozoa of tilapia (*O niloticus*) submitted to different distributions.

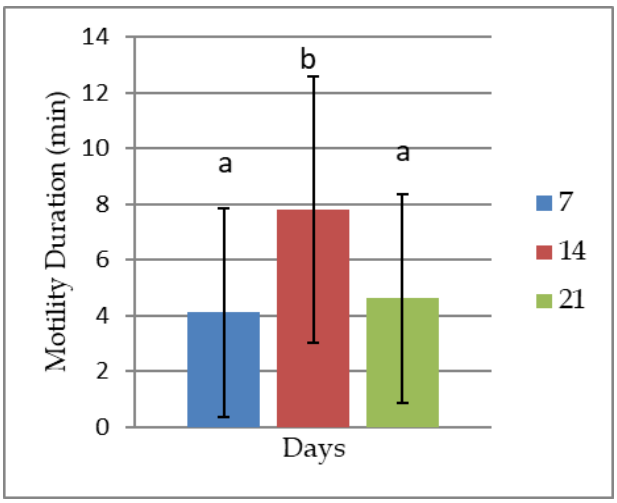

Figure 5. Motility duration (min) for tilapia semen (*O. niloticus*) submitted to different days of placement.

DISCUSSION

The extrusion is an importante step in fish reproduction development. It is posible to note that females without hormonal induction at 7 days of placement showed greater facility of extrusion when kept together with males with visual and chemical contact, and when placed together with males the inductor hCG, while separated from males without the inductor were difficult to extrude. However, in this period at least 66, 67% of the females were considered of ease extrusion.

There is a tendency of tilapia to respond to hormonal induction as a reproductive behaviour regulator depending on the environmental conditions in which they are and the variety providing guarantees on the facility of extrusion of gametes, for possible manipulation and fertilization in genetic selection programs

(SOUZA, 2016).

The hormonal induction provided an increase of 16, 66% in facility of extrusion in the females that were separated. The increase of that variable in fish might be related with the ability of hCG to increase the rates of spawning and the interactions during visual and chemical contact between males and females kept in the same environment, stimulating the release of pheromones in water, which in a turn accelerate ovarian follicular maturation. (ORFÃO, 2013 e SOUZA et al, 2016) state that inductor hormones has influenced in an increase of spawning weight. During interactions, tactile information, visuals, chemical, acoustics and electrical can be transmitted, emphasizing that the reproductive physiology of fish can be affected in several sensory ways. This information can have different effects according to each specie (HONJI, 2020).

The females studied for 21 days as for extrusion, presented greater facility when they were together with males maintaining visual and chemical contact, with administration of hormone and difficult when they are separated independent of induction. In this period, at least 81, 82% of females which were together with males with hormonal induction were considered of ease extrusion.

There was an increase in spawning weight by administration of hormone, environment and days of placement. This results confirm the answers found in several research works with tilapia, of their high prolificacy as long as the physical environmental conditions and physiological are guaranteed. (NETO et al., 2014; SOUZA et al., 2016)

When it comes to tilapia production, it is observed that some environmental factors are more important in relation to others, thus the better results are obtained considering the environment and genetics together, offering the best conditions to obtain the highest performance.

Reports on diameter evaluations are important as they influence in the larvae survival, this being a good sign for the increase in energy reserve quantity (LIMA e BARBOSA; SOUZA et al., 2016), in this regard the findings in this work are of big contribution for tilapia reproductive development, ensuring quality spawning and in survival of future generation.

The size increase of egg in fish can be influenced by physical and physiological conditions of the reproducers, due to age, selection criteria and

environmental conditions (ANDRADE et al., SILVA., NETO et al., 2015). However, the variation in size of the eggs found in this study, may be related to different gonadal maturation stages in which the females were found. It was observed in inducted females and separated from males, oocyte larger in diameter than those reported by Andrade et al. (2015) referred to as normal diameters which vary between 2 and 7,9 mm and 2,0 and 3,0mm. According to writers like Souza at al., (2016), the diameter of oocytes can be used as quality parameter of spawning the larger the diameter the better is the quality of this spawning and the future offspring.

The possibility to control the reproductive cycle of the organisms submitted to confinement conditions is one of the factors of greater importance to secure success of fish farming, since induction to spawning in fish using hormones has guaranteed the obtention of fertile eggs, allowing embryonic description of several species, in some cases, greater production of healthy families (ORFÃO et al., 2013 e MARQUES et al., 2016).

According to (FELIZARDO et al., 2012 e DIEMER, 2014), in order to obtain high rate of fertilization, it is necessary that the semen and oocytes are in perfect conditions of use, being that for spawning, one of the main characteristics is the germinal visicula in peripheral position, probably the lower temperature may have influenced in findings of peripheral position of germinal visicula , for although the females showed extrusion facility, the of quality oocytes were not different between the treatments.

Hormonal induction in tilapia with LH-RH, dopamine antagonist and hCG is a method which has been used as a way to guarantee the best moment for fertilization of gametes in genetic selection programs (ALVARENGA, 2015 e SOUZA et al., 2016). However, for these procedures to bring us good results it is essential that the gametes are ready so that they follow their process until obtaining healthy families.

Fish semen can be evaluated in different ways, depending on the specie, individual, collection method, among other factors. It is important that evaluation are made that will allow to establish right proportions, to fertilize a certain quantity of oocyte in an efficient way, allowing to obtain a total of larvae needed by batch.

Hormonal treatment administered some hours before collection is frequently used to increase the semen volume. This method, also facilitate the collection because stimulates testicular hydration which causes increase of semen volume, meanwhile,

simultaneously, reduction of spermatic concentration occur (SILVA, 2015 e DALMASS et al., 2016). As observed in this study, the volume values and concentration were influenced by days and by different distributions.

The values found in the present work were also observed by Souza et al., (2016), who found 0, 9 mL for GIFT variety and 0, 69 mL for UFLA variety with administration of hormone. According to the writers like SILVA (2015), the seminal volume is an important factor in reproductive process and that in both migratory species in the production season and in animals induced with hormones is quite variable. The success of reproduction depends on the maximum use of available gametes, which means to fertilize the highest number of oocytes with the fewest quantity of spermatozoa (SENAR, 2017). Thus seminal volume is a variable which must be analyzed together with concentration, motility duration and seminal motility, this because the more the variables, probably the better the quality of semen will be. According to (DE OLIVEIRA et al., 2015 e FERNANDES et al., 2020) there is a big variation in the data of spermatic concentration in fish in general, seeming to have specific specie influence, having also big individual differences, bringing up different information within the same species. Report of Honji et al., (2017), the LH is responsible for the rupture of the cysts and spermiation, thus it is probable that the concentration increase in semen of separated tilapias may be related to hormonal induction associated with high concentrations of FSH and LH. However, Honji et al., (2017) & Souza et al., (2018) quote that the increase of serum concentrations of FSH e LH would act increasing the spermatic concentrations, what was demonstrated in this study.

The rates of motility duration found in this study are higher than those found by Pereira et al., (2016), with rates of 96% in 46sec. These results ensure the semen quality. This increase is probably due to the use of hormonal induction in this work, bearing in mind that other writers do not use this protocol, as the animals squirted naturally. Writers such as Paulino et al., (2016), observed 2, 55 duration for tilapia. For motility and semen integrity there was no significant differences for the environment, induction and days. This parameter is used to evaluate the spermatic quality and is directly related to spermatic motility (SOUZA, 2018).

Regarding the quantity of normal spermatic cells or integrity found in this work, the values reflect the normal progression of spermatogenesis. Souza et al.,

(2018) found 50% of normal cells for the specie, without hormonal induction. Our integrity findings of spermatozoa are within the limits for the use artificial fertilization of fish. According to Souza et al., (2018)., the critical percentage for use in fertilization is below of 50% of normal spermatozoa, since this technic involves a high rate of spermatozoa in controlled conditions.

CONCLUSIONS

At 14 days, with the animals distributed together and hormonal induction with hCG were obtained the best results contributing to reproductive synchronization of females. As found for males, at 14 days, the animals distributed separately were those which showed the best results contributing to reproductive synchronization of tilapia. The administration of the hormone hCG has proved to be efficient for synchronization of the reproductive activity in tilapia, contributing positively in production and in genetic improvement of the specie.

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