PHOTOPERIODIC EFFECTS IN BLOOD GLUCOSE, CORTISOL, HEMATOLOGICAL PARAMETERS AND REPRODUCTIVE INDEXES OF GIFT LINEAGE REVERSED MALE TILAPIA

EFEITO DO FOTOPERIÓDO NA GLICOSE SANGUÍNEA, CORTISOL, PARÂMETROS HEMATOLÓGICOS E ÍNDICES REPRODUTIVOS DA LINHAGEM GIFT TILAPIA MASCULINA

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ABSTRACT: The objective of this study was to evaluate the influence of the photoperiod on the physiological, hematological and reproductive parameters of tilapia males of the GIFT lineage. The treatments differed in the simulations of emeral photoperiods T1 = 0L: 24E T2 = 24L: 0E, T3 = 12L: 12E. Each aquarium was considered an experimental unit. In the present study, tilapia males under different photoperiods did not present significant differences in gonad weight and gonadosomatic index. At the end of the experiment, there were also no significant differences in hepatosomatic indexes (IHS) of Tilapia males under different treatments. In the present experiment, it was observed that the 0L: 24E treatment obtained a shorter testicular length in relation to the other treatments. In addition, the Tilapia submitted to 24L: 0E presented significant difference for the glucose levels, and did not present significant difference for cortisol levels and survival rate compared withthe other treatments. There were no statistical differences for hematological values. Exposure to different light regimes has shown that for GIFT tilapia, the photoperiod that promotes better reproductive index and well-being conditions is the photoperiod (12L: 12E).

KEYWORD: Stress. Blood. Reproduction. Light. Dark.

INTRODUCTION

The movements of rotation and translation of the Earth make the organisms that inhabit it subject to cyclical changes of environmental factors. All life forms respond to the sun, the moon and the seasonal cycles, being denominated biological clock or circadian rhythm. Most biochemical, physiological, and behavioral events of living beings are rhythmic. The factors external to the clock of rhythmic nature that influence the [illegible] are the main synchronizers of daily and annual rhythms (MENDONÇA et al., 2009; NAVARRO and NAVARRO, 2012, NAVARRO et al, 2013a).

Brazil has a high potential for fish farming because of its environmental conditions favorable to the high diversity of native species with potential for cutting and ornamentation and the vast availability of water resources. Aquaculture minimizes environmental impact as it reduces extractive fishery and provides standardization and regularization of fish supply (NAVARRO et al., 2012).

The influence of the environment on animals is notorious for its development, survival and reproduction. The photoperiod corresponds to one of several environmental stimuli and is related to the duration of light time over a day. The intensity and time increase of this light change with the seasons of the year and the climate of the region (BEZERRA et al, 2008; BLANCO-VIVES et al., 2009 and 2010; NAVARRO et al., 2013b). [illegible]or is an important [illegible] equalization of the whole creation according to the seasons of the year.

Tilapias consist of a large number of species, a group of exclusively African fish, of the Cichlidae Family. In Brazil, commercial strains have different origins: Tilapia-de-Bouaké originated from the Ivory Coast and introduced in 1971, the Chitralada or Thai lineage, developed in Japan and improved in Thailand, imported into Brazil in 1996, And the GIFT (Genetically Improved Farmed Tilapia) lineage developed in the Philippines

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(FÜLBERET al., 2009). Tilapia production in Brazil accounts for 45% of the continental fish production, forming an expressive source of resources and the need for technification in its production.

Studies aimed at clarifying the influence of photoperiod on the growth and physiological patterns of exotic species in Brazil are scarce in the literature. Thus, the objective of this study was to evaluate the effect of different photoperiods on the hormonal, hematological and reproductive indexes of tilapia males from the GIFT lineage.

MATERIAL AND METHODS

Animals and treatment

The experiment was carried out at the Aquaculture Laboratory of the Faculty of Agronomy and Veterinary Medicine of the University of Brasília - UnB, lasting for 60 days. The study was approved in the animal ethics committee of the University of Brasília (CEUA / UnB protocol number 43058/2012).

A total of 150 GIFT Tilapia fingerlings (*Oreochomisniloticus*), sexually reversed males with 17α-methyl-testosterone hormone, with an initial weight of 5.28 ± 1.8g and a length of 5, 5 ± 0.8 cm at a density of 0.65 L / fish, were employed. Weight and total length data for each fish, of different treatments, were measured using a caliper rule (or Pachymeter) and precision scale (0.001g). The fish were conditioned and distributed in 15 aquariums of 65 liters each, in a recirculation system in which aquarium water was collected and filtered through a mechanical and biological filter installed in a water tank of 500 L for later use, in order to maintain the water quality of the system. The experimental design was completely randomized, with three treatments and five replicates. The treatments differed in the simulations of photoperiods T1 = 0L: 24E, T2 = 24L: 0E, T3 = 12L: 12E. Each aquarium was considered an experimental unit.

The control of the light hours provided during the experiment was done by automatic timers that switched off and on the bench lights during periods stipulated using fluorescent lights (20W) with luminous intensity of 1200 Lux measured with the use of a digital lux meter model CT-1330B.

The treatments were isolated with black PVC material to avoid the incidence of light coming from other light sources during the dark period of each treatment in order to ensure that the light supply was deliveredduring the stipulated periods for each treatment.

The temperature was checked daily at 8:20 p.m. and 5:20 p.m. The dissolved oxygen, pH, ammonia and nitrate were checked every 7 days. The levels of oxygenation were maintained with the aid of aerators and measured through a digital oximeter. The pH was measured through the pH meter, the temperature using a digital thermometer,the ammonia and the nitrate were measured through the water quality kit of the company alfakit.

Food management was carried out using a commercial ration containing:

28% PB and 3,100 kcal ED / kg; Moisture (Max.) 120.0g; Crude Protein (min.) 280.0g; Fiber (Max) 40.0 g Ethereal Extract 60.0 g Lysine 10.0 g Methionine (min) 4.500.0 mg Calcium (max) 30.0 g; Phosphorus (min) 5,0000,0g; Vitamin C (min) 200 mg and premix 0.5%.

The food was divided into three meals a day. The ration was provided at 8:20 a.m., 1:20 p.m. and 5 p.m. After one hours of each feeding, the leftovers were removed from the aquaria so that all animals were fed the same amount of feed and did not compromise the quality of the water. Biometrics were performed every 15 days in order to adjust the feed provided.

Biometrics and Reproductive Indices

At 60 days of experiment, specimens of Tilapia males, fasted for 24 hours, were removed and anesthetized with a solution containing 65 ppm of Eugenol for desensitization to the gonad collection procedure. The animals were then weighed and measured for standard and full length values. Later, the gonads were collected and measurements of the length and width of the gonads were performed in order to calculate the gonadossomatic index (GSI) and the hepatosomatic index (HSI), which indicate the gonadal development and nutritional status of the fish, respectively, using The following formulas:

IGS = PG / PC x 100, where IHS = PF / PC x 100, where

PG = gonad weight PF = liver weight

PC = body weight PC = body weight

Biochemical analyzes

Glucose

Blood samples were taken at the beginning of the experiment and at the end of the experiment. To collect blood, 10 fish from each treatment were used, previously anesthetized with a solution containing 65ppm of Eugenol. Blood samples were collected by intracardiac puncture using 3 mL

syringes. A minimum volume of 0.4 mL of blood per fish was collected.

An aliquot of collected blood was used to quantify blood glucose through digital glucose apparatus: ACCU - CHEC SOFTCLICK - UNITED STATES. Most of the collected blood was deposited in heparin-containing eppendorfs and wrapped in ice until samples of all fish were collected. The samples were centrifuged at 11000 rpm for 2 minutes. The heparinized plasma (supernatant) was then removed with a digital pipette and stored in labeled eppendorfs for further hormonal analysis. All plasma material was frozen in a freezer at -20 ° C until the time of analysis.

Cortisol

For determination of cortisol, the plasma was analyzed by the ELISA technique (Kit CORTISOL 96T, ELISA - HUMAN © 2013 Abnova Corporation).

Hematological Analyzes

After 60 days of experiment, three fish were collected per replicate. The fish were previously anesthetized with a solution containing 65ppm of Eugenol to perform intracardiac puncture blood collection using 1 mL syringes, bathed with 3% EDTA anticoagulant.

For the determination of hematocrit, the microhematocrit technique was used (MOURENTEET al., 2005). The collected blood was placed in capillary tubes and then taken to the centrifuge for 2 min at 11,000 rpm. The reading was performed on a standard scale and the values expressed in%. Among the cells of the red series, hemoglobin was quantified according to Collier (1944); Mean corpuscular volume, mean corpuscular hemoglobin concentration, according to Costa et al., (2014) and total erythrocyte count in the Neubauer chamber.

An aliquot of blood was used in the preparation of blood extensions stained by the method of Rosenfeld (1947) for evaluation of cell morphology. Cell counts were performed using an automated method performed by the hematological analyzer ABCVet® (HORIBA -JAPAN), controlled by microprocessor used for the in-vitro diagnostic test of compound blood samples, a fully automated device with an internal dilution system,working with sample handling in an open tube with the need to prior homogenize the sample with aspiration of 12 µl blood by the apparatus of each tube analyzed. Among the leukocytes, lymphocytes and monocytes were differentiated.

Statistical analyzes

Normally distributed data were analyzed using one-way variance analysis (ANOVA) coupled with Duncan tests. Statistical analyzes were performed using SAS software version 6.02.

RESULTS AND DISCUSSION

The water quality parameters presented the following mean values: 21.74 ± 0.43 ° C for initial temperature, 29.50 ± 0.03 ° C for ambient temperature, 5.5 for pH; 6.1 ± 0.36 mg.L^{-1}, dissolved oxygen, value of 0.35 for ammonia and 0.10 for NO_2 according to Navarro et al., (2012).

After the 60 days of experiment, no significant differences ($p > 0.05$) were observed in the final weight and total length between treatments (Table 1). Other studies corroborate the results of the present study, where manipulation of the photoperiod did not influence the total length and body weight of Linguado (*Veraspermoseri*) (AMANO et al., 2004) and Nile Tilapia (*Oreochromisniloticus*) (Campos -Mendoza et al., 2004). However, the lower growth revealed in the treatment of Light-24 may be a reflection of the higher level of cortisol presented in this treatment in relation to the others.

According to Pickering (1993), the catabolic effect of cortisol on chronic stress is responsible for the suppression of growth. In addition, the results of the present study differed from other studies, where researchers found a positive relationship between exposure to continuous light, body weight, somatic growth, and food intake. This relationship was observed in Atlantic salmon (*Salmosalar*) (Atlantic salmon) in Atlantic cod, (*Gadusmorhua*) (Tarangeret al., 2006) in Arinca (*Melanogrammusaeglefinus*) (RED et al., 2004). The results obtained in this work are shown in Table 1. Navarro et al. (2015) observed that light continues (24L: 0D) in Nile tilapia increases somatic growth.

In the current study, tilapia males under different photoperiods did not present significant differences in gonad weight and gonadosomatic index ($p > 0.05$). This may be justified by the little influence of the photoperiod on the maturation process of this species compared withother environmental variables, or by the use of a lineage of animals that have a later gonadal maturation associated to the insufficiency in the time of animal exposures to different regimes of light.

Contrasting these results, several studies point to the interference of the photoperiod with these quantitative reproductive variables. Long

photoperiods such as continuous light regimes tend to promote suppression of gonadal maturation and redirection of food energy from gonadal development to somatic growth (BOEUF; LE BAIL, 1999). Rad et al. (2006) observed that Nile Tilapia fingerlings maintained under continuous light showed reduced gonadal maturation and lower IGS values, followed by higher somatic growth.

In the case of Salmonidae and temperate fish subjected to long or continuous light photoperiods, developmental delay and gonadal maturation were also observed, associated with lower IGS values (DAVIE et al., 2007, TARANGERET al., 2006). Millaet al. (2009) noticed lower IGS values in Perca (*Percafluviatilis*) maintained under constant photoperiod conditions (12L: 12E) for 3 months. However, Miranda et al. (8L: 16E) showed lower IGS values in relation to fish exposed to longer photoperiods (16L: 8E).

At the end of the experiment, no significant difference in IHS ($p > 0.05$) was detected for tilapia males under different treatments, suggesting that manipulation of the photoperiod probably did not influence energy expenditure for somatic development (Table 1). However, in the study by Tarangeret al. (2006) Atlantic cod males and females (*Gadusmorhua*) under continuous light regime (LL) presented higher values of IHS than the natural light regime (LN), indicating that the LL group invested less energy for reproduction. Ribeiroet al. (2006) also found differences in the IHS, in Sagüiru-tailed-yellow (*Steindachnerinainsculpta*), that decreased from the rest phase to the maturation stage.

The photoperiod is responsible for gonadal maturation by exerting direct action on the hypothalamic-pituitary-gonadal axis of the teleost fish, stimulating or inhibiting the production of gonadotrophin releasing hormone (GnRH), pituitary hormones (FSH and LH) and other hormones that modulate reproduction and maturation of the gametes (AMANO et al., 2004) . In the current experiment it was observed that the animals of the 0L: 24E treatment obtained a shorter testis length in relation to the other treatment (table 01). Exposure of this species to a long dark period associated with a probable release of higher levels of hormone melatonin may probably directly or indirectly inhibit the growth of the gonad. Most studies involving photoperiod and fish reproduction point to a relationship between the photoperiod and the hypothalamic-pituitary-gonadal axis, reflected by changes in spermatogenesis and changes in the levels of pituitary hormones and steroids linked to reproduction.

Table 1. Final weight (g), total length (cm), hepatosomatic index (IHS), gonad weight (PG), gonadosomatic index (IGS), gonad length and gilate width of tilapia males as a function of photoperiod.

Treatment	24L:0E	12L:12E	0L:24E	P
Final weight (g)	42,13± 17,5	43,65± 13,40	44,68± 19,42	P>0,05
Total length (cm)	13,41±1,61	13,64±1,66	13,76±1,89	P>0,05
Hepatosomatic index (IHS)	2,64±1,63	2,03± 0,68	2,31±0,53	P>0,05
Weightofgonad (PG)	0,11±0,05	0,11±0,06	0,12±0,05	P>0,05
Gonadsomatic index ((IGS)	0,28± 0,12	0,27± 0,09	0,34± 0,12	P>0,05
Lengthofgonad (cm)	3,95±1,51a	3,16±0,77a	3,05±0,70b	P<0,05
Width of the gonad (cm)	0,16±0,17	0,12±0,04	0,11±0,03	P>0,05

Averages on the same line with different envelopes are significantly different according to Duncan's test with a mean ± SEM 5% level.

After 60 days a significant difference ($p < 0.01$) in the glucose levels was verified, and the treatment with light-24 showed lower glycemic levels (Table 2). Probably, the intense swimming activity during the whole period of continuous light led to a greater exhaustion of the fish, followed by a greater energetic demand and lactate glucose break by the process of anaerobic glycolysis in the white muscle in order to attend the motor activities and not the ones responsible for the growth processes. This lower glycemic level in the light-24 treatment can also be explained by the higher levels of cortisol in this treatment that tends to reduce serum levels of plasma glucose (NIKAIDOET al, 2010). Navarro et al. (2014) also observed a significant decrease in glucose levels in the light-24 treatment for the Tambiúlambari female (*AstyanaxBimaculatus*).

The higher luminosity in 24L: 0E treatments followed by increased cortisol levels did not interfere in the survival rate between treatments. The results showed that an increase in swimming activity may lead to a higher probability of encounters between fish and therefore greater susceptibility of fish to attack each other.

Table 2. Plasma glucose concentration, cortisol and survival rate of tilapia as a function of the photoperiod.

Treatment	24L:0E	12L:12E	0L:24E	P
Glucose (mg/dL)	29,72± 1,93a	53,33± 3,96b	61,28± 5,19b	P<0,05
Cortisol (ng/mL)	8,70 ± 0,04a	6,50 ± 0,04b	7,20 ± 0,08c	P<0,05
Survival Rate%	96±5,00	83±29,66	80± 15,71	P>0,05

Averages in the same row with different envelopes are significantly different according to Duncan's test with a level of 1% Mean ± SEM.

In the present study, the hemogram values were not statistically disparate for the different treatments for hematocrit, total protein, total erythrocyte count, Hbb, WBC + plaq, monocytes and platelets (Table 3). The values were within the reference standards,with similar averages obtained by Tavares-Dias and Faustino (1998) and Ueda et al. (1997) with *Oreochromis*. Hematological values in fish can be influenced by exogenous and endogenous factors such as by the action of cortisol (RANZANI-PAIVA, 1991).

The lower leukocyte level of the 24L: 0E treatment, although not statistically different, can be attributed to a stress situation, since this treatment obtained a higher plasma level of cortisol (Table 1 and 3) in relation to the 12L / 12E treatment. Tavares-Dias &Faustino (1998) and Ueda et al. (1997) reported that the stress hormone provoked a situation of physiological fish exhaustion, with reduced blood cell production, and these results were also observed in Sea Bass by (BAGNIET al., 2005). The response to stress is marked by the decrease in the fish resistance to diseases because of the depletion in the number of leukocytes, lymphocytopenia, according to Mazeuaudet al. (1977).

To other hematological values of lymphocytes and segmented, no significant differences between treatments were observed. *Oreochromisniloticus x O. aureusSteindachner*, (Nussey et al., 1995) and *Oreochromismossambicus* (Tavares) values were found to be higher than those discovered by (SHIAU; LUNG, 1993) with *Oreochromisniloticus x O. aureus.*

Table 3. Percentage of Hematocrit, Total Protein, total count of erythrocytes, HgB, Wbc + plaq (WBC count and platelets), WBC, platelets, monocytes, lymphocytes, segmented (SEGM) of tilapia males as a function of photoperiod.

Treatment	24L:0E	12L:12E	0L:24E	P
Hematocrit	23,16± 8,04	26,27± 5,47	22,75± 7,02	P>0,05
Total Protein (PT)	3,06± 0,67	3,50± 0,42	3,05± 1,16	P>0,05
Total erythrocyteHgB count	1,42± 0,26	1,51± 0,37	1,19± 0,50	P>0,05
	4,20± 1,73	6,09±1,08	4,62± 1,43	P>0,05
WBC + PLAQ	73,16± 18,49	80,44± 43,86	73,28 ± 92,63	P>0,05
WBC	56,36± 13,48	68,84± 34,94	63,66± 67,29	P>0,05
MONO	12,4± 6,06	14,4± 8,96	10,625± 3,99	P>0,05
LINF	70,8± 21,62	74± 25,46	79,75± 10,66	P>0,05
PLAQ	16,8± 17,58	11,6± 17,68	9,62± 11,27	P>0,05
SEGM	22,95± 12,31	15,04± 13,86	35,97± 27,28	P>0,05

Averages on the same line with different envelopes are significantly different according to Duncan's test with a mean ± SEM 5% level.(PT) - Total Protein,(WBC) - Total white blood cell count,(RBC) - Total red cell count,(MONO) - Monocyte count,(PLAQ) - Platelet Count,(SEGM) - Segmented lymphocyte count

CONCLUSION

This work concludes that, based on the aforementioned conditions, the photoperiod regime (12L: 12E) promoted better welfare and reproductive indexes in tilapia of the GIFT lineage.

RESUMO: O objetivo deste estudo foi avaliar a influência do fotoperíodo nos parâmetros fisiológicos, hematológicos e reprodutivos de machos de tilápia da linhagem GIFT. Os tratamentos diferiram nas simulações dos fotoperíodos emeral T1 = 0L: 24E T2 = 24L: 0E, T3 = 12L: 12E. Cada aquário foi considerado uma unidade experimental. No presente estudo, os machos de tilápia sob diferentes fotoperíodos não apresentaram diferenças significativas no peso gonadal e no índice gonadossomático. Ao final do experimento, também não houve diferenças significativas nos índices hepatossomáticos (IHS) dos machos de tilápias sob diferentes tratamentos. No presente experimento, observou-se que o tratamento 0L: 24E obteve menor comprimento testicular em relação aos demais tratamentos. Além disso, a tilápia submetida a 24L: 0E apresentou diferença significativa para os níveis de glicose, e não apresentou diferença significativa para os níveis de cortisol e sobrevivência em comparação com os demais tratamentos. Não houve diferenças estatísticas para valores hematológicos. A exposição a diferentes regimes de luz mostrou que, para a tilápia GIFT, o fotoperíodo que promove melhor índice reprodutivo e condições de bem-estar é o fotoperíodo (12L: 12E).

PALAVRAS-CHAVE: Estresse. Sangue. Reprodução. Luz. Escuridão.

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