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Full Length Article

Influence of Extended Photoperiod on All Male Nile Tilapia (*Oreochromis niloticus*) Production, Differential Gene Expression and Growth Rate

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Abstract

The effects of an extended photoperiod (18 h light or 12 h light), using a combination of natural and artificial light sources, on the differential gene expression and growth rate of Nile tilapia *Oreochromis niloticus* L. were evaluated. Four groups of all males tilapia (n=10) with initial mean body weight of 102.25 g were reared in an aquaria with two replications for each treatment. The experiment was conducted during a period of 35 days, growth rate and water quality (dissolved oxygen, pH, nitrite, nitrate) were measured weekly, temperature and solar radiation were recorded daily. At the end of the experiment we used a zebrafish genome array to study differential expression over 14,900 transcripts and to test the hypotheses that an extended photoperiod stimulates growth rate and causes differential gene expression in tilapia. Fishes in the extended photoperiod group (Ep) were susceptible to show higher growth rate (GR) compared with the standard photoperiod group (Sp; P = 0.001). Food conversion efficiency in the Ep was superior than the Sp. Differential expression of genes related to transcriptional regulation for biological function was detected using zebrafish GeneChip® (Affymetrix) microarrays, this could explain how the fish alters their metabolism as response to this phenomena. © 2015 Friends Science Publishers

Keywords: Combined light sources; Differential gene expression; Extended photoperiod; Growth rate; *Oreochromis niloticus*

Introduction

Aquaculture has been growing steadily in recent times as an excellent source of high-quality protein. It is growing at 9% every year and it is estimated to grow up to 41% (53.6 million tonnes) of the fish production worldwide by the year 2020 (Krishen *et al*., 2009). Now-a-days several poor countries, mainly in Asia, score close to 85% aquaculture production worldwide. Researchers focused on Nile Tilapia because of its quick reproduction rate, tolerance to hard environments, endurance to disease and the possibility to be cultured under diverse farming systems (Yosef, 2009; Soto-Zarazúa *et al*., 2010a).

Males are preferred because they grow almost twice as fast as females, which may be caused by a sex-specific physiological growth capacity, female mouth-brooding or the more aggressive feeding behavior of males (Hafeez-ur-Rehman *et al*., 2008). Expected survival for all-male culture is 90% or greater. About six months are required to produce 500 g fish from 50 g fingerlings, with a growth rate of 2.5 g/day (Fortes, 2005).

Male Tilapia production has an economic importance to its producers and sellers, with an increase in employment in the sector outpacing world population growth and employment in traditional agriculture is a crucial source of income and livelihood for hundreds of millions of people around the world (Soto-Zarazúa *et al*., 2011). It may play an important role to secure food for the general population together with an excellent source of high-quality protein (FAO, 2012; Soto-Zarazúa *et al*., 2010b).

The behavior and growth performance of Nile tilapia, *O. Niloticus* have been evaluated under different factors like water contamination, ammonia and temperature (El-Sherif and El-Feky, 2009), feeding components (Al-Asgah *et al*., 2011), environmental color (Elnwishy *et al*., 2012) and photoperiod (El-Sayed and Kawanna, 2007). The photoperiod also has an influence over other traits like seed production (Ridha and Cruz, 2000), which support further investigation over the effect of extended photoperiod. All these studies have been performed in order to optimize the cultivation of the specie. These variable culture conditions may influence the growth performance of Tilapia (Elnwishy *et al*., 2007), since an artificial environment may vary from the natural habitats of fish that may cause negative effects on fish feeding activity, health and growth (Elnwishy *et al*., 2012).

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The study of the influence of gene expression in performance traits like growth rate, feed conversion efficiency, body conformation, disease resistance and sex determination is an opportunity to meet the demands of fish production while ensuring profitability (Liu, 2007). Genetics is a field of study in constant development. Functional genomics is an emerging discipline that is used to study gene expression, genomic controls and transcriptional profiles and it is being applied to cultured fish species. The challenge is to determine what each gene does in terms of the development and physiological functioning of the organism (Murphy, 2002). Gene expression in Tilapia could be useful for the rapid assessment of growth rate under different feeding and temperature regimes (Vera Cruz *et al*., 2006) and to provide information about mechanisms that play a role in stressful situations like suboptimal feeding (Villarroel *et al*., 2005).

Microarrays analysis of gene expression profiles has become the most widely used functional genomics tool in fisheries (Nielsen and Pavey, 2010). The use of microarrays to study gene expression in closely related species has shown that expression differences can be estimated without discernible loss of information (Oshlack *et al*., 2007), another applications indicate that use of the zebra fish microarray is a valid way to examine other fish (and mammalian) species (Malakar *et al*., 2013). These studies allow the use of the zebrafish GeneChip® (Affymetrix) to study differential gene expression in Tilapia. Now with the genetic maps of Nile Tilapia available (Kocher *et al*., 1998; Lee *et al*., 2005; Guyon *et al*., 2012) and the Ensembl genomic databases (*Oreochromis niloticus*; Orenil 1.0) the need is to assign biological function to the genes.

It is surmised that a combined light source to obtain an extended photoperiod may affect all male *Tilapia* production phenotypically and genotypically. The objective of this study was to compare the effect of natural and extended photoperiod regimes, using combined light sources, on male Tilapia production.

Materials and Methods

Experimental Details

The experiment was carried out inside a polyethylene greenhouse in Amazcala, Queretaro state, Mexico. (Longitude (decimal degrees): -100.265833, Latitude (decimal degrees): 20.703333).With an average height of 1920 meters above the sea. Four cubic tanks with a capacity of 320 L were used. Blue canvas was chosen inside tanks because it promotes growth rate according to Elnwishy *et al*. (2012). 20% of total water was replaced daily with fresh water at the same temperature and continuously aerated to maintain optimum water quality (Van Rijn *et al*., 2006). Tanks were instrumented with temperature (°C) and solar radiation (W/m^2) sensors, recorded in a data logger (Watch Dog). Dissolved oxygen (DO, saturation %), pH, nitrates (NO_3-N) and nitrites (NO_2-N) were measured weekly to evaluate water quality (HACH laboratory equipment). The photoperiod was extended to 16 h light (16 L) in accordance with the observations of Vera Cruz and Brown (2009) to compare with natural photoperiod (12 L). Two white spiral light bulbs (1400 lm each, Havells) fixed at 50 cm above water level that did not affect water temperature. Photoperiod was controlled by 24 h time controllers (Tecno Lite).

Fish Management

Forty all male *O. niloticus* with a mean mass of = 102 g (± 2.5) were arbitrarily separated into four groups (ten fish each) and each group was deposited in a 320 L tank. The experiment design was two groups with 1 replica per group. The group with the extended photoperiod regime got 16 h of light and 8 h of darkness (Ep), and the standard photoperiod was 12 h of light and 12 h dark (Sp). Each group was fed once a day in the morning with a commercial pelleted diet. This was to make certain that the rise in growth rate was because of bigger food conversion efficiency (Vera Cruz and Brown, 2009). Every week the feed amount was adjusted consistently with mass gain, feed conversion efficiency (FCE), fish mass-specific growth rates (GR) and relative growth were calculated weekly as follows:

FCE = mass gained/total amount of feed consumed

$GR = 100\ [(\ln M_f - \ln M_i)\ t^{-1}]$

$RG = 100\ (M_f - M_i)\ M_i^{-1}$

M_f = final body mass (g)

M_i = initial body mass (g)

t = growth time (days)

After five weeks, four specimens from each tank were taken randomly, individual mass was calculated and samples were snap frozen on liquid nitrogen to obtain total RNA.

RNA Isolation

Total RNA from tissue samples was isolated using the extraction method of Chomczynski and Sacchi (1987) with the TRIzol ® reagent. Integrity of each RNA sample was evaluated by two methods: Image analysis with agarose gel electrophoresis using Gel Red™ stain and Molecular Imager ® Gel Doc™ XR + System with Image Lab™ Software to capture the image. RNA was quantified and purity was assessed by spectrophotometry (A260:A280 ratio, NanoDrop® 2000 Thermo Scientific). Every sample got a ratio of ~2.0 which is generally accepted as "pure" for RNA (Bustin 2000, 2002).

Differential Gene Expression Analysis

Oligonucleotide microarrays (Zebrafish Gene 1.0 ST Affymetrix) and Affymetrix GeneChip system for examining gene expression profiles were used. One pool of each condition was labeled with biotin and hybridized to separate arrays (Lockhart *et al*., 1996). Affymetrix fluidic

station 450 was used for the wash and stain operation of the arrays. The image was captured with the Scanner Unit model "3000 7G".

The R software, available from the Bioconductor Project (http://www.bioconductor.org), was used to preprocess and normalize data before comparison and to evaluate differential expression between pool samples. Data normalization was made using the Robust Multichip Average (RMA) method (Irizarry *et al*., 2003). It consists of three steps: a background adjustment, quantile normalization and finally summarization. All differential expression where selected from a > 2 fold change using the oligo (Carvalho and Irizarry, 2010) and limma (Smyth, 2005) packages.

Statistical Analysis

Mass from four samples from each tank was obtained to calculate mean mass from each group. In the statistical analysis of variance to assess the same treatments in different tanks, no significant evidence to suggest that the results were due to the tanks was found. Based on the statistical analysis of variance was obtained enough evidence to say that the differences between the replicas were because of the treatment and not by the different ponds. Impact of photoperiod on the growth rates (GR) and feed conversion efficiency (FCE) of the fish were evaluated using one-way ANOVA. All statistical analyses were done using Minitab 16 software (www.minitab.com).

Results

Water Quality

Temperature data (Fig. 1) indicates that the light used to obtain an extended photoperiod (Fig. 2) did not affect the temperature in the water; differences in the measurement (±1°C) were due to the sensors themselves. The concentrations for the different water quality parameters (average, minimum and maximum) were within safe ranges and acceptable limits for tilapia production (Soto-Zarazúa *et al*., 2010a) throughout the experiment. Average concentrations for extended photoperiod were 85% DO, 8.2 pH, 2.4 mgL^{-1} NO_3-N and 0.106 mg L^{-1} NO_2-N and for natural photoperiod were 101% DO, 8.5 pH, 2.75 mg L^{-1} NO_3-N and 0.125 mg L^{-1} NO_2-N. These values were comparable with those reported under similar systems (Van Rijn *et al*., 2006; Soto-Zarazúa *et al*., 2010a). Average concentration of nitrates and nitrites are not in the toxic range and, therefore, would have no adverse effect on growth rate. Increased oxygen consumption in ponds with extended photoperiod suggests an accelerated metabolism which favors higher growth rate.

Growth Rate and Feed Conversion Efficiency

Mean mass were distributed equally between treatments at the beginning of the research. At the end of experimental period (35 days) fish in the extended photoperiod group (Ep) showed higher specific growth rate mean (Fig. 3: GR 0.849% per day, STD=0.0902%), higher feed conversion efficiency mean (FC 0.65) and higher relative growth mean (RG 37.75%) than the normal photoperiod group (Fig. 3: GR 0.619% per day, STD=0.1249%, FC 0.53, RG 21.56%) this difference between the two groups was significant ($P<0.05$, Media Ep=137.66 g, STD=4.34 g, Media Sp=126.96 g, STD=5.52 g).

Differential Gene Expression

We detected differential expressed genes in our conditions using oligonucleotide microarrays. All differentially expressed genes were selected with a fold change > 2X and a p-value < 0.05. Overall, we found 35 genes being up-regulated by the altered photoperiod (Fig. 4). We searched for the human orthologs for those genes with ENSEMBL Genome Browser (www.ensembl.org). Genes on the list with a relevant known function (commercial, medical, DNA recognition) are listed in Table 1.

Discussion

The photoperiod recommended for seed production coincided to obtain bigger growth rate where breeders under longer photoperiod exhibited higher fecundity (Ridha and Cruz, 2000), which indicates that an extended photoperiod will increase growth rate without affect other performance traits (egg qualities and fecundity) according to the results of Onumah *et al*. (2010). Specific growth rate was significantly affected by the extended photoperiod. Fish in the extended photoperiod group had faster growth rate. This could be due to a raise in appetite and feed intake, better feed efficiency and/or elevated digestibility according to Vera Cruz and Brown (2009). Data suggest that the efficiency and hence the economics could be improved with an extended photoperiod regime. Photoperiod manipulation to improve production is a better option than the normally used approach by increasing the amount of food, since this increases the residual food which leads to deteriorating water quality and lower system performance (Jobling, 1998).

One of the differentially expressed genes observed in this study was the CD22 which is a B-cell receptor CD22-like. The CD22 is a marker that is expressed in B cell development, and is expressed in mature B prior to their differentiation into plasma cells and identified in lung cancer, as well as lymphocytic leukemia, in chronic nonspherocytic hemolytic anemia, and systemic lupus erythematosus (Tuscano *et al*., 2012). The arglu1b gene, arginine and glutamate rich 1b, which is also a mediator and transcribes estrogen receptors and growth of cells of lung cancer is also expressed (Zhang *et al*., 2011). Odorant receptor, LOC100149406, is also expressed and is a promoter in the activation of B cells, which influence cancer diseases.

Table 1: Differentially expressed genes with a commercial, medical and/or genetic relevance

Expressed genes	Function
CD22	Cluster of differentiation 22 expressed in lung cancer, identified new antigen (Toscano *et al.*, 2012)
Odorant receptor (LOC100149406)	Extracellular calcium receptor, promotes activation of human B cells (Hammond *et al.*, 2007)
tlr19	Toll like receptors of the innate immune response (Krutzik and Modlin, 2004)
arglu1b arginine and glutamate rich 1b	Interacts with the mediator (MED1) and transcribed to estrogen receptors and growth of breast cancer cells (Zhang *et al.*, 2011)
TRAP1 TNF Receptor-associated protein 1	Mitochondrial protein belonging to the family of heat shock, antigen for cancer therapy
atp1a1a.4 ATPase	Enzymes which catalyze the decomposition of adenosine triphosphate (ATP into adenosine diphosphate (ADP)
zgc: 162358, zgc: 175284, ZFP37 (51 of 55), ZNF519 (2 of 2)	Zinc finger proteins cleave the DNA and have multiple functions such as recognition of DNA and RNA packaging

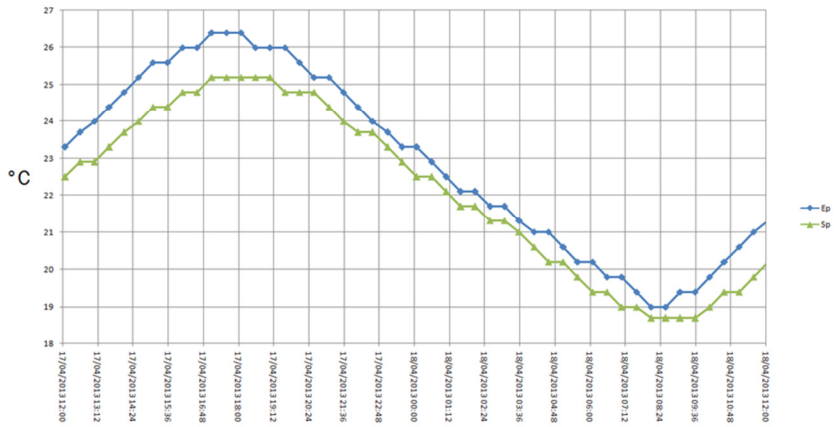

Fig. 1: Temperature under water throughout the day inside tanks. The difference between tanks temperatures is due by the calibration of the sensors itself

Temperature in both tanks varies in similar way without been influenced by the white bulb lamps.

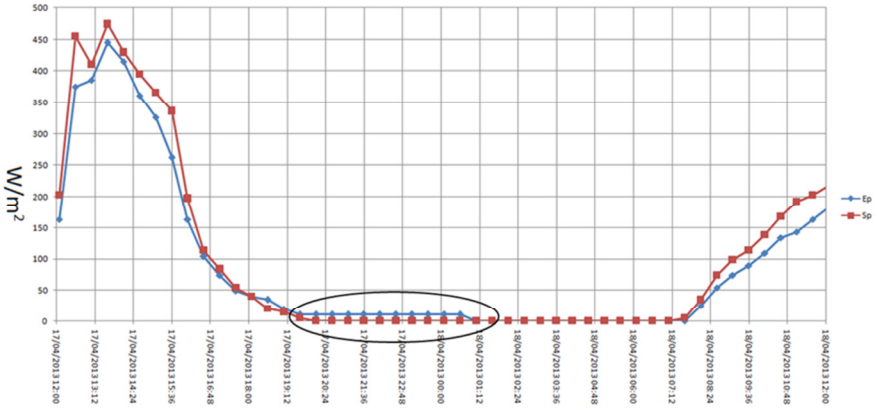

Fig. 2: Extended photoperiod using white light bulbs

The extended photoperiod is seen in this graphic from 19:00 to 01:00, it could be seen from Fig. 1 and 2 that the extended photoperiod does not affect the temperature inside the tank

TRAP1, TNF receptor-associated protein 1, is used as an antigen for cancer therapy and is a gene which is fully identified in periodic syndrome associated to TNF receptor, known by the acronym TRAPS (Liu *et al*., 2010). The TNF-receptor-associated periodic syndrome (TRAPS) is an autosomal dominant auto-inflammatory disorder, characterized by recurrent febrile attacks and localized inflammation. TRAPS is caused by mutations in the gene encoding the TNF Receptor Super Family 1A (TNFRSF1A) on chromosome 12p13.

Other genes found were atp1a1a.4 ATPase, zgc: 162358, zgc: 175284, ZFP37 (51 of 55), ZNF519 (2 of 2) enzymes that catalyze the breakdown of ATP to ADP, and have multiple functions of DNA recognition, RNA

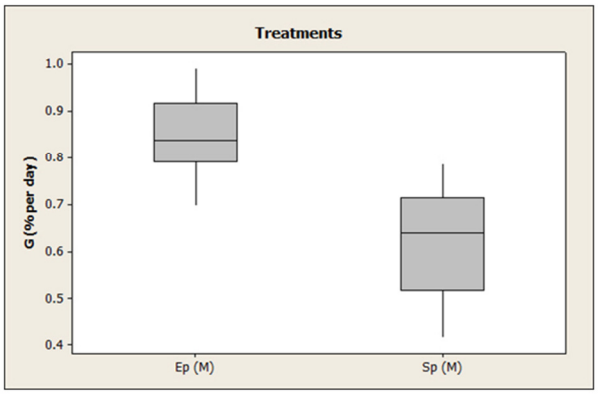

Fig. 3: Specific growth rate comparison between extended photoperiod and standard photoperiod groups

Boxplot extended photoperiod group shows higher mean and less deviation than the standard photoperiod group. Box plots are useful for identifying outliers and for comparing distributions. The box plot is a graphical representation of data that shows a data set's lowest value, highest value, median value, and the size of the first and third quartile

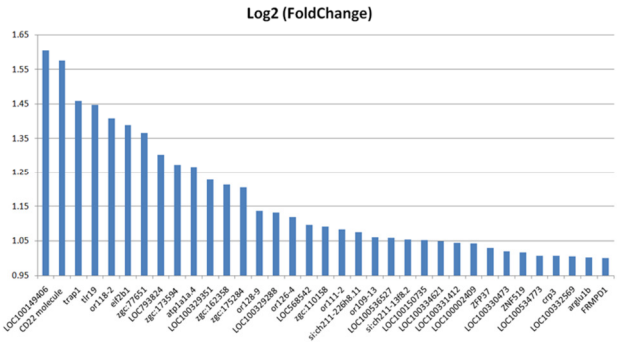

Fig. 4: Differentially expressed genes with a fold change >2X in the expended photoperiod group

The selected genes have relevant functions identified and some are commercially important. The fold-change of a gene probe is the ratio of the mean of the experimental values divided by the mean of the control set of data for that gene probe. Ratio target gene in experimental/control = fold change in target gene/fold change in reference gene

packaging, transcriptional activation, regulation of apoptosis, protein folding and assembly and lipid binding (Laity *et al.*, 2001). Extended photoperiod promoted differentially gene expression which can be used as antigens in cancer disease treatment investigations, its prevention and control.

Conclusion

Extended photoperiod has a benefic impact on growth rate and differential gene expression compared to those reared under natural photoperiod. There is a potential for maximizing tilapia production thorough photoperiod manipulation. Furthermore a differential gene expression was found. However, supplementary studies are required to test the effect of extended photoperiod on longer periods of time in commercial tilapia production.

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