Latin American Journal of Aquatic Research

E-ISSN: 0718-560X

lajar@pucv.cl

Pontificia Universidad Católica de Valparaíso

Chile

Ramírez, Eduardo; López-Cardiel, Josué; Lezama, Carlos; García-Márquez, Luis; Borja-Gómez, Imelda; Tintos-Gómez, Adrián

Effect of Passiflora incarnata (L) extract on gonadal maturation in young tilapia (Oreochromis sp.)

Latin American Journal of Aquatic Research, vol. 45, núm. 5, noviembre, 2017, pp. 908-914

Pontificia Universidad Católica de Valparaíso

Valparaíso, Chile

Lat. Am. J. Aquat. Res., 45(5): 908-914, 2017
DOI: 10.3856/vol45-issue5-fulltext-5

Research Article

Effect of *Passiflora incarnata* (L) extract on gonadal maturation in young tilapia (*Oreochromis* sp.)

Eduardo Ramírez[1,3], Josué López-Cardiel[1], Carlos Lezama[1,3], Luis García-Márquez[2] Imelda Borja-Gómez[1] & Adrián Tintos-Gómez[1,3]

[1]Facultad de Ciencias Marinas, Universidad de Colima, México, Manzanillo, México
[2]Facultad de Medicina Veterinaria y Zootecnia, Universidad de Colima, Tecomán, México
[3]Departamento de Estudios para el Desarrollo Sustentable de la Zonas Costeras
Universidad de Guadalajara, Municipio de Cihuatlán, México
Corresponding author: Adrián Tintos-Gómez (atintos48@gmail.com)

ABSTRACT. The effect of *Passiflora incarnata*'s extract (PE) on gonadal maturation in young tilapia (*Oreochromis* sp.) was evaluated by administering feed supplemented with PE during the first 90 days immediately after yolk sac assimilation. One hundred and fifty fishes with 0.01 ± 0.003 g average body weight were randomly distributed in ten tanks with the following duplicated treatments: commercial feed (NAT), NAT with 60 mg kg^{-1} of 17-MT (MET), and NAT supplemented with 31.10 mg, 62.30 mg and 124.60 mg of PE g^{-1} (P1, P2, P3 respectively). There were no significant differences ($P > 0.05$) in the average body weight or in the male-female sex ratio between the experimental treatments (P1, P2, P3) and the control (NAT). Statistical differences in the gonadosomatic index (GSI) and the percentage distribution of gonadal maturation stages (PDGMS) were observed in females of P2 and P3. With respect to the females of NAT, the treatments P2 and P3 presented a lower GSI and a lower percentage of females in stage III (GSI: 1.11 ± 0.88, 1.04 ± 0.99 and 1.71 ± 0.72; PDGMS: 45, 30 and 80%, respectively). No significant differences in GSI or PDGMS were observed in the males. The results suggest that the observed differences in GSI and PDGMS in females are unrelated to anti-nutritional effects. Instead, these differences could be due to a possible antiestrogenic effect attributed to the possible anti-aromatase action of some of its compounds. Future research focusing on the use of PE for reproductive control in tilapia is suggested.

Keywords: *Passiflora*, *Oreochromis*, tilapia, gonadal maturation, gonadosomatic index.

INTRODUCTION

Tilapia is the most cultivated group of fishes around the world, with the second highest production, after carp (FAO, 2014). For the commercial production of tilapia it is vital to have a thorough knowledge of the basic aspect of their biological cycle, specifically reproductive aspects. Studies performed over the last decades have provided insight into these aspects and assisted in their integration into cultivation practices (Das *et al.*, 2012). The success of tilapia culture results from the excellent adaptation of this group of fishes to industrial culture conditions but their reproductive precocity requires control measures to prevent the diversion of energy from muscle production to reproduction (Das *et al*., 2012; El-Greisy & El-Gamal, 2012). Some of the most frequently used reproductive control measures involve the creation of monosex cultures through sex reversal, interspecific hybridization, and manual sex selection. Although monosex cultures can be either male or female, males are preferred because they reach larger sizes in a shorter amount of time (Camacho-Berthely *et al*., 2000; Mlalila *et al.*, 2015). The most commonly used technique for the creation of monosex cultures is known as sex reversal, which consists in the oral administration of the synthetic hormone 17-α-methyltestosterone (17-α-MT) incorporated into feed and supplied during the first days of life after yolk sac assimilation (7 to 10 days after hatching). During this period the gonads are stimulated to develop into testes both in males and genotype females due to the masculinizing action of this synthetic hormone (Green & Teichert-Coddington, 2000; El-Greisy & El-Gamal, 2012). In the first days of

Corresponding editor: Cesar Lodeiros

fish life the gonads are in a period of non-differentiation (bipotential) and are susceptible to external influences that can alter the normal process of sexual differentiation (Devlin & Nagahama, 2002). Over the natural process of sexual differentiation, the gonads develop following the chromosomal arrangement and under the action of the hypothalamic-pituitary-gonadal axis, which regulates the process through production of sex steroids, mainly testosterone and estrogen. Testosterone is the hormonal inducer of testicular development and estrogen induces development of the ovaries (Bhandari *et al*., 2006; Guiguen *et al*., 2010). Although testosterone has a masculinizing action and estrogen feminizing, estrogen is synthesized from testosterone through the action of aromatase (Devlin & Nagahama, 2002).

The most recommended treatment for sex reversal is oral administration of powdered fish feed (40 to 50% of total protein) supplemented with 30 to 60 mg of 17-α-MT kg^{-1} of feed during the first 25 to 60 days of life, which does not represent a health risk for the organisms during the cultivation period or for consumers. Over the four weeks of treatment, 0.2 mg of 17-α-MT $fish^{-1}$ is utilized and the estimated residual 17-α-MT concentration in the body of treated tilapias does not exceed 1.5-10 μg $fish^{-1}$ day^{-1}. Hormone levels in treated tilapia begin to normalize 5 days after the end of treatment, decreasing to less than 10 ρg of 17-α-MT g^{-1} of muscle 6 to 50 days after the end of treatment, therefore product safety is assured after the 120 clearance days suggested by the Food and Drug Administration (FDA) (Mlalila *et al*., 2015). An average adult male has the ability to remove up to 10 mg of androgen per day. This guarantees the elimination of any residual 17-α-MT that could be found in the muscle after the clearance period recommended by the FDA, ensuring the safety of the product offered on the market (Green & Teichert-Coddington, 2000; El-Greisy & El-Gamal, 2012; Megbowon & Mojekwu, 2014; Mlalila *et al*., 2015). Despite the effectiveness and safety of this technique, in the last decades' herbal extracts have been proposed for use as active agents to control reproduction in the cultivation of tilapia, in order to find more economically accessible alternatives for aquaculture development (Gabriel *et al*., 2015). The use of herbal extracts as sex reversal inducers in fish has a variable efficiency percentage, most often no more than 80%, depending on the plant used, concentration of active ingredients, the species studied, and the developmental phase of the organism during application (Ghosal & Chakraborty, 2014; Ghosal *et al*., 2016). This places the alternative at a disadvantage in comparison to the 97% sex reversal of the conventional method (El-Greisy & El-Gamal, 2012). In search for alternatives to maintain reproductive control, studies have been carried out such as those by Jegede (2010) and Kushwaha (2013) to evaluate the effect of *Hibiscus rosa-sinensis* (2.0 g kg^{-1} feed) and *Aloe vera* extract (4.0 g kg^{-1} feed), respectively, on the development and gonadal maturation of *Oreochromis niloticus*. Both studies used mixed reproductive populations with the respective diets. Testicular and ovarian structural degeneration was found in the population fed a diet supplemented with extract of *H. rosa-sinensis*, and degeneration of sperm and follicular cells was seen in the population fed a diet supplemented with *A. vera*. Therefore, it is possible to conclude that both extracts can be used for reproductive control of *O. niloticus*. Obaroh & Nezh-Achionye (2013) evaluated the effect of extracts of *Azadirachta indica* and *Mangifera indica* in the feed of *O. niloticus*, using mixed juvenile populations, fed for 56 days. Those authors found that production of offspring was inhibited in populations administered feed supplemented with these extracts (1.0 and 2.0 g kg^{-1}, respectively), concluding that *A. indica* and *M. indica* can be used to control the prolific production of offspring in tilapia cultures.

The *Passiflora* genus is a widely distributed group of plants, of which a wide variety of bioactive effects have been reported, mainly anxiolytics, sedatives, and antispasmodics, among others (Dhawan *et al*., 2004; Ingale & Kasture, 2014). The most studied species in this respect is *P. incarnata*, although research into its effects on reproduction is limited. Dhawan & Sharma (2003) evaluated the effect of the methanolic *Passiflora* extract on the deterioration of some sexual parameters (fecundity and sperm count) in male rats exposed to Δ9-tetrahydrocannabinol (Δ9-THC), finding that by administering feed supplemented with 10 mg kg^{-1} of Δ9-THC + 20 mg kg^{-1} *P. incarnata* methanolic extract for 30 days, fecundity and sperm count were not altered, in contrast with the positive control (Δ9-THC 10 mg kg^{-1}) where these parameters were below normal values. Furthermore, Bacchi *et al*. (2013) evaluated the effect of prenatal exposure to *P. incarnata* extract in rats, analyzing the sexual behavior of males 22, 35 and 75 days after birth, and found that males exposed to 300 mg kg^{-1} (body weight) through maternal pathways showed less sexual competition than the controls. The effect of *P. incarnata* on reproduction is attributed to the possible anti-aromatase action of some of its compounds, which include: BZF (tri-substituted benzoflavone moiety), vitexin, iso-vitexin and orentine, among others (Dhawan & Sharma, 2003; Dhawan *et al*., 2004; Masteikova *et al*., 2008). There is no research evaluating the effect of *P. incarnata* on reproductive parameters of tilapia, so the present study assessed the effect of *Passiflora* ethanolic extract on the gonadal

maturation in tilapia, in order to document any possible antiestrogenic action and discuss its potential use as a reproductive control agent in tilapia cultures.

MATERIALS AND METHODS

The experiment was carried out over 90 days between September and December 2013, in the Aquaculture and Ecotoxicology laboratory of the Faculty of Marine Sciences of the Universidad de Colima, Campus Universitario El Naranjo, in the municipality of Manzanillo, Colima, Mexico. The fish were donated by the Centro Acuícola de Occidente S.A. de C.V., located in the same municipality. The *P. incarnata* raw extract (PE), was donated by Rosa Elena Dueñas S.A. de C.V. (REDSA) laboratory (density 0.9870-1.0442 g cm^3, alcohol density 0°-12° LG, relative pH 5.0-6.36, total solids 2.63-19.35%). One hundred and fifty young and sexually undifferentiated tilapias (0.01 ± 0.003 g average body weight), randomly distributed in each of ten 500 L tanks, from which five groups were formed in duplicate after a 24 h acclimatization period.

i. NAT (negative control): commercial feed Nutripec ®.
ii. MET (positive control): hormonated commercial feed Silver Cup ®.
iii. P1 (experimental diet; low dose): 12.52 g PE kg^{-1} of NAT.
iv. P2 (experimental diet; medium dose): 25.05 g PE kg^{-1} of NAT.
v. P3 (experimental diet; high dose): 50.1 g PE kg^{-1} of NAT.

Water quality conditions remained within the optimal range according to Camacho-Berthely *et al.* (2000) through the application of daily water changes constant aeration and total disposal of solid waste.

The organisms were fed during the first 90 days of life after assimilation of the yolk sac in two distinct feeding regimens. During the first 30 days of the experiment the organisms were given powdered feed (53% protein, 15% fats, 0.33 mm size for treatments NAT, P1, P2, P3, and 52% protein, 14% fats, 0.33 mm size for the treatment MET) at 20% of body weight in 8 daily servings at 1 hour intervals. From day thirty one, the treatment consisted of feed pellets (43% protein, 15% fats, 1.5 mm for each treatment) at a rate of 15% body weight (El-Greisy & El-Gamal, 2012) in 4 servings daily at 2 hour intervals over the remaining 60 days of treatment. At the end of the experimental period, 20 fish were randomly caught in each treatment per replicate, anesthetized with clove oil, weighed and then killed by hypothermia. The gonads were removed from the body cavity, weighed for the Gonadosomatic Index (GSI) calculation using the equation GSI = (gonad weight/total weight) × 100, and preserved in a buffered formalin solution for histological analysis to determine sex and degree of sexual maturity. The gonads were processed according to the paraffin embedment technique where 5 µm thick sections were obtained from the paraffin blocks and stained with hematoxylin-eosin (HE). The gonadal maturation indices were determined based on those reported in Peña-Mendoza *et al*. (2011).

A one-way ANOVA of weight and GSI was carried out. Likewise, a non-parametric (Q-Cochran) analysis of the percentage of males and females in each treatment, as well as the percentage distribution in the different stages of maturation (PDGMS) was performed. Subsequently a *post-hoc* (Tukey HSD) test was applied.

RESULTS

There was no significant difference ($P > 0.05$) between the treatments mean weights as well as between the percentages of males found in the treatments, except between MET and NAT (Table 1). There was no significant difference in the GSI between males, whereas the GSI in females of treatments P2 and P3 presented statistically significant differences with respect to females of NAT (Table 2).

Gonad weights varied between 0.02 g to 0.60 g among males and 0.03 g to 0.60 g among females (Fig. 1). Histological evaluation of the gonads revealed that all organisms, including both males and females, were distributed within stages II and III of gonadal maturation (Table 2, Figs. 1, 2).

DISCUSSION

The results on the sex ratio indicates that *Passiflora* extract does not have a significant effect on the percentage of males (Table 1), which explains why there are no previous studies reporting the use of *Passiflora* flour or extract as a sex reversal inducer in fish (an exhaustive search was made in the CONRICyT; Science Direct; Google Scholar databases).

No lesions were observed on the gonads under the macroscopic and histological assessments (Figs. 1, 2), nor was there a statistically significant difference in the mean weight attained in the experimental treatments with respect to the controls (Table 1). Therefore, it can be inferred that PE does not have toxic effects on the gonadal cells or anti-nutritional consequences for young tilapia. This supports the findings of Boll *et al*.

Table 1. Percentage of males, survival and mean final body weight. Values in the same column and with the same superscript did not present significant statistical differences ($P < 0.5$). US: undefined sex, n: number of organisms.

Treatment	Males/ Females (%)	Survival (%)	Weight (g)		
			Females	Males	n
MET	95/5[b]	90[a]	US	16.01 ± 1.28[a]	20
NAT	55/45[a]	90[a]	15.34 ± 1.42[a]	15.36 ± 1.30[a]	20
P1	50/50[a]	87[a]	15.15 ± 1.64[a]	14.95 ± 1.52[a]	20
P2	50/50[a]	95[a]	14.10 ± 1.48[a]	14.33 ± 1.56[a]	20
P3	55/45[a]	84[a]	13.68 ± 1.95[a]	14.46 ± 2.09[a]	20

Table 2. Gonadosomatic index and percent distribution of gonadal maturation, by sex and treatment. Values in the same column and with the same superscript present significant statistical differences from NAT ($P < 0.5$). N/O: no observation, F: female, M: male, n: number of organisms.

Treatment	GSI		Gonadal maturation stage								n	
			I		II		III		IV		20	
	F	M	F	M	F	M	F	M	F	M	F	M
MET	N/O	1.67 ± 0.89[a]	N/O	N/O	N/O	20	N/O	80	N/O	N/O		19
NAT	1.71 ± 0.72[a]	1.58 ± 0.71[a]	N/O	N/O	20	25	80	75	N/O	N/O	9	11
P1	1.35 ± 0.68[ab]	1.41 ± 0.73[a]	N/O	N/O	30	30	70	70	N/O	N/O	10	10
P2	1.11 ± 0.88[b]	1.45 ± 1.01[a]	N/O	N/O	55[a]	35	45[a]	65	N/O	N/O	10	10
P3	1.04 ± 0.99[b]	1.52 ± 0.97[a]	N/O	N/O	70[a]	35	30[a]	65	N/O	N/O	9	11

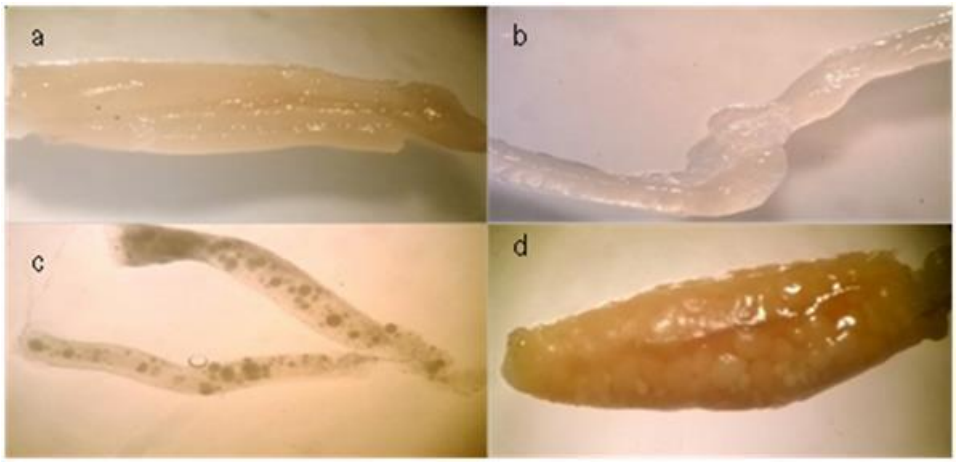

Figure 1. a) Male gonad belonging to the first group with weights between 0.2 to 0.6 g, b) male gonad belonging to the second group with weights between 0.02 to 0.15 g, c) female gonad belonging to the first group with weights between 0.03 a 0.15 g, d) female gonad belonging to the second group with weights between 0.23 to 0.6 g.

(2014), who reported no negative effects from *P. incarnata* in pregnant and lactating rats, and Devaki *et al*. (2012) who reported that *P. incarnata* does not produce toxic effects in rats; however, Boeira *et al*. (2010) reported that *P. alata* produces mild genotoxic damage and inhibits weight gain in mice.

The GSI and PDGMS of the females in P2 and P3 treatments showed statistically significant differences with respect to those obtained from the NAT females. They showed lower GSI values for P2 (1.11 ± 0.88) and P3 (1.04 ± 0.99) compared to NAT (1.71 ± 0.72), a PDGMS of 45% in state of maturation for P2, and 30% in III for P3, while NAT presented a PDGMS of 80% in III. The males presented no significant differences between these same parameters (Table 2). The organisms used in the present study were able to reach their first sexual maturity, and the GSI and the gonadal maturity rate were observed to be directly related, with a lower GSI, corresponding to an earlier stage of maturity. It was also found that organisms with a lower

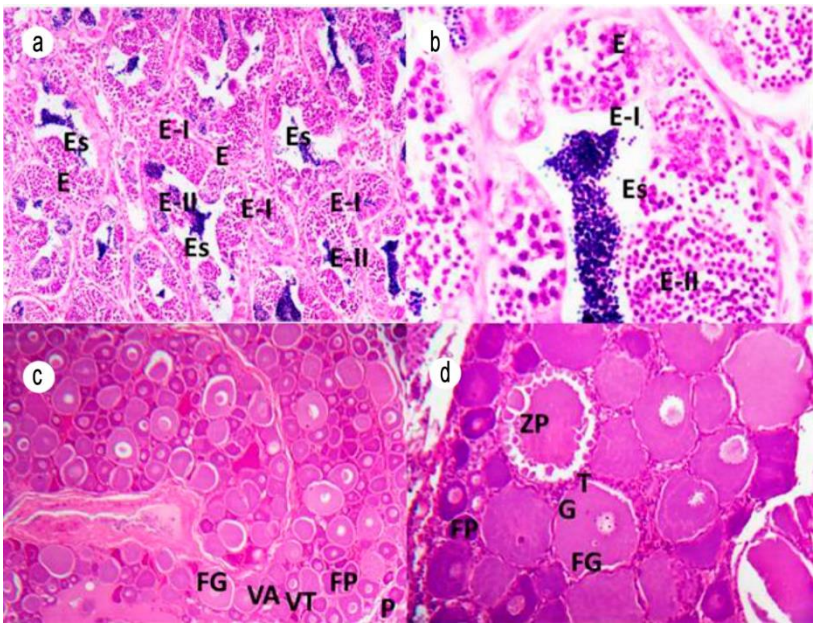

Figure 2. Tilapia gonads in maturation state II and III. Histological sections: a) testis in stage II (beginning of maturation), where spermatogonia (E), primary (EI) and secondary (E-II) spermatocytes are observed in the spermatic ducts, and sperm formation (Es) begins in some ducts, b) stage III (maturation phase) where the same structures of stage II are observed, in addition to most efferent ducts being full of mature spermatozoa (Es), c) ovary in stage II of development, where large follicles (FG), small follicles (PF), follicles in previtellogenesis (P), early vitellogenesis (VT) and advanced vitellogenesis (VA) are observed, d) stage III, where the pelucida zone (ZP), granand teak cells (T) are observed. Hematoxylin-eosin staining (10x) (40x).

GSI presented macroscopic differences. In general, both males and females presented thin and translucent gonads, while males with a higher GSI presented opaque cream-colored gonads with a sinuous appearance, which could be due to the increase of spermatozoa and the corresponding increase of testicular lumen. Likewise, females with a larger GSI presented orange cream-colored gonads with the appearance of granular sacs, corresponding to the greater presence of follicles in advanced and late vitellogenesis (Figs. 1, 2).

The results show that PE has no effect on gonadal maturation in males, although there was a decrease in the percentage of females that reached stage III of gonadal maturation with respect to the natural response control (NAT). This could indicate that the effect of PE is related to the synthesis or bioactivity of estrogen (Dhawan *et al*., 2004), possibly an inhibitory effect that impedes gonadal maturation in females due to the fact that there was no similar effect in males. Masteikova *et al*. (2008) reported that the ethanolic extract of *P. incarnata* (50.0 g of crude material 100 mL^{-1}, percolation method) contains: Chlorogenic acid (284 ± 7.0 μg mL^{-1}), Hyperoside (178 ± 8 μg mL^{-1}), Isovitexin (4963 ± 142.0 μg mL^{-1}), Caffeic acid (175 ± 11.0 μg mL^{-1}), Quercetin (7 ± 0.5 μg mL^{-1}), Luteolin (21 ± 4.0 μg mL^{-1}), Orentin (3299 ± 89.0 μg mL^{-1}), Rutin (164 ± 9.0 μg mL^{-1}), Scutelarein (42 ± 3.0 μg mL^{-1}), Vicenin (6102 ± 45.0 μg mL^{-1}), and Vitexin (1436 ± 21.0 μg mL^{-1}). Bioactive effects have been reported among them, such as antioxidant, anti-inflammatory, anti-cancer and anti-aromatase (Olthof *et al*., 2001; Gülçin, 2006; Kim *et al*., 2011; Marrassini *et al*., 2011; An *et al*., 2012; Choo *et al*., 2012; Lu *et al*., 2012; Lee *et al*., 2013; Shu-Yao *et al*., 2013). However, *in vivo* effects (especially anticancer and anti-aromatase), of both flavonoid rich extracts and purified extracts are still debated (Saarinen *et al*., 2001; Moon *et al*., 2006; Genoux *et al*., 2011; Lephart, 2015). The works by Dhawan & Sharma (2002, 2003); Dhawan (2003) and review by Dhawan *et al.* (2004), suggest that the compound known as BZF, which is described as a tri-substituted α-naphthoflavone isolated from the methanolic extract of *P. incarnata*, has a strong anti-aromatase activity by restoring libido decline, sperm count and fertility in male rats exposed to substances such as cannabinoids, alcohol or nicotine. It is thought that the BZF activity mechanism acts through the inhibition of enzyme aromatase, which would prevent the transformation of testosterone to estrogen. This

appears to correlate with the results obtained in this study, because aromatase is the key enzyme in ovarian development and maturation in fish, and is involved in the biosynthesis of ovarian estradiol (Guiguen *et al*., 2010). The biosynthesis of ovarian estradiol is a key step in the process of vitellogenesis, in which the oocyte increases in size and develops its structures (Mañanós *et al.*, 2008), and correlates with the results obtained from the GSI and PDGMS in the females of treatments P2 and P3. Those females presented a lower GSI, more females in previtello-genesis (stage II), and fewer females in late vitellogenesis (stage III) with respect to the females of NAT, which suggests that the PE has a possible antiestrogenic and/or anti-aromatase effect. These results may serve as a basis for future research focused on the use of PE on gonadal maturity development, as well as on reproduction control in tilapia culture.

ACKNOWLEDGMENTS

To the Faculty of Marine Sciences, Universidad de Colima, for the facilities used to develop this work. To Rosa Elena Dueñas S.A. de C.V. Laboratory, for the donation of the *Passiflora* extract. To Dr. Fernando Vega Villasante, for his valuable observations. To Dip. Fed. Francisco Alberto Zepeda González (LXIII legislature), for his support in the purchase of some reagents necessary for the development of this research.

REFERENCES

An, F., G. Yang, J. Tian & S. Wang. 2012. Antioxidant effects of the orientin and vitexin in *Trollius chinensis* Bunge in D-galactose-aged mice. Neural Regen Res., 7: 2565-2575.

Bacchi, A.D., B. Ponte, M.L. Eieira, J.C.C. de Paula, S.F.P. Mesquita, D.C.C. Gerardin & E.G. Moreira. 2013. Developmental exposure to *Passiflora incarnata* induces behavioural alterations in male progeny. Reprod. Fert. Develop., 25: 782-789.

Bhandari, R.K., M. Nakamura, T. Kobayashi & Y. Nagahama. 2006. Suppression of steroidogenic enzyme expression during androgen-induced sex reversal in Nile tilapia (*Oreochromis niloticus*). Gen. Comp. Endocr., 145: 20-24.

Boeira, J.M., R. Fenner, A. Betti, G. Provensi, L. de A. Lacerda, P. Barbosa & S.M.K. Rates. 2010. Toxicity & genotoxicity evaluation of *Passiflora alata* Curtis (Passifloraceae). J. Ethnopharmacol., 128: 526-532.

Boll, K., M. Bortolasci, C.C. Zaminelli, T. Veríssimo, L.F. Bacchi, A.D. Higachi & L. Moreira. 2014. *Passiflora incarnata* treatment during gestation and lactation: toxicological and antioxidant evaluation in wistar dams. Braz. J. Pharm. Sci., 50: 353-359.

Camacho-Berthely, E.C. Luna-Romo & M.A. Moreno-Rodríguez. 2000. Guía para el cultivo de tilapia *Oreochromis* sp. (Gunter, 1984). SEMARNAP, México, 136 pp.

Choo, C.Y., N.Y. Sulong, F. Man & T.W. Wong. 2012. Vitexin and isovitexin from the leaves of *Ficus deltoidea* with *in-vivo* α-glucosidase inhibition. J. Ethnopharmacol., 142: 776-781.

Das, R., M.A. Rather, N. Basavaraja, R. Sharma & U.K. Udit. 2012. Effect of non-steroidal aromatase inhibitor on sex reversal of *Oreochromis mossambicus* (Peters, 1852). Israeli J. Aquacult. Bamigdeh, 64: 1-9.

Devaki, K., U. Beulah, G. Akila & A. Gopalakrishnan. 2012. Effect of aqueous extract of *Passiflora edulis* on biochemical and hematological parameters of Wistar albino rats. Toxicol. Int., 19: 63-70.

Devlin, R.H. & Y. Nagahama. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, and environmental influences. Aquaculture, 208: 191-364.

Dhawan, K. & A. Sharma. 2002. Prevention of chronic alcohol and nicotine-induced azospermia, sterility and decreased libido, by a novel tri-substituted benzoflavone moiety from *Passiflora incarnata* Linneaus in healthy male rats. Life Sci., 71: 3059-3069.

Dhawan, K. & A. Sharma. 2003. Attenuation of chronic Δ9-THC-induced decline in sexuality in male rats by a novel benzoflavone moiety of *Passiflora incarnata* Linn. Brit J. Pharmac., 138: 117-120.

Dhawan, K. 2003. Drug/substance reversal effects of a novel tri-substituted benzoflavone moiety (BZF) isolated from *Passiflora incarnata* Linn. a brief perspective. Addict Biol., 8: 379-386.

Dhawan, K., S. Dhawan & A. Sharma. 2004. *Passiflora*: a review update. J. Ethnopharmacol., 94: 1-23.

El-Greisy, Z.A. & A.E. El-Gamal. 2012. Monosex production of tilapia, *Oreochromis niloticus* using different doses of 17α-methyltestosterone with respect to the degree of sex stability after one year of treatment. Egypt J. Aquat. Res., 38: 59-66.

Food and Agriculture Organization (FAO). 2014. El estado mundial de la pesca y la acuicultura. FAO, Roma, 253 pp.

Gabriel, N.N., J. Qiang, M.D. Kpundeh & P. Xu. 2015. Use of herbal extracts in controlling reproduction in tilapia culture: trends and prospects -a review. Israel J. Aquacult., 67: 29 pp.

Genoux, E., E. Nicolle & A. Boumendjel. 2011. Flavonoids as anticancer agents: recent progress and state of the art. Curr. Org. Chem., 15: 2608-2615.

Ghosal, I. & S.B. Chakraborty. 2014. Effects of the aqueous leaf extract of *Basella alba* on sex reversal of Nile tilapia, *Oreochromis niloticus*. IOSR-JPBS, 9(2): 162-164.

Ghosal, I., D. Mukherjee, C. Hancz & S. Bhusan. 2016. Production of monosex Nile tilapia, *Oreochromis niloticus* by dietary and immersion treatment with *Basella alba* leaves and *Tribulus terrestris* seeds. IJFAS, 4(1): 358-363.

Green, B.W. & D.R. Teichert-Coddington. 2000. Human food safety and environmental assessment of the use of 17α-Methyltestosterone to produce male tilapia in the United States. J. World Aquacult. Soc., 31(3): 337-357.

Guiguen, Y., A. Fostier, F. Piferrer & C. Chang. 2010. Ovarian aromatase and estrogens: a pivotal role for gonadal sex differentiation and sex change in fish. Gen. Comp. Endocrinol., 165: 352-366.

Gülçin, İ. 2006. Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid). Toxicology, 217: 213-220.

Ingale, S.P. & S.B. Kasture. 2014. Antioxidant and antiparkinsonian activity of *Passiflora incarnata* leaves. Orient. Pharm. Exp. Med., 14: 231-236.

Jegede, T. 2010. Control of reproduction in *Oreochromis niloticus* (Linnaeus, 1758) using *Hibiscus rosa-sinensis* (Linn.) leaf meal as reproduction inhibitor. J. Agricult. Sci., 2: 149-154.

Kim, S., J. Um, S. Hong & J. Lee. 2011. Anti-inflammatory activity of hyperoside through the suppression of nuclear factor-κB activation in mouse peritoneal macrophages. Am. J. Chin. Med., 39: 171-181.

Kushwaha, P. 2013. Effects of *Aloe vera* (Liliaceae) on Gonad development in Nile tilapia, *Oreochromis niloticus* (L.) during intensive aquaculture. IJFAS, 1: 56-60.

Lee, C., S. Shen, Y. Lai & S. Wu. 2013. Rutin and quercetin, bioactive compounds from tartary buckwheat, prevent liver inflammatory injury. Food Funct., 4: 794-802.

Lephart, E.D. 2015. Modulation of aromatase by phytoestrogens. Enzyme Res., 2015: 11 pp.

Lu, D., L. Yang, F. Wang & G. Zhang. 2012. Inhibitory effect of luteolin on estrogen biosynthesis in human ovarian granulose cells by suppression of aromatase (CYP19). J. Agr. Food Chem., 60: 8411-8418.

Received: 1 October 2015; Accepted: 6 May 2017

Mañanós, E., N. Duncan & C. Mylonas. 2008. Reproduction and control of ovulation, spermiation and spawning in cultured fish. In: E. Cabrita, V. Robles & P. Herráez (eds.). Methods in reproductive aquaculture: marine and freshwater Species. CRC Press, Taylor & Francis Group, Boca Raton, pp. 3-80.

Marrassini, C., R. Davicino, C. Acevedo, C. Anesini, S. Gorzalczany & G. Ferraro. 2011. Vicenin-2, a potential anti-inflammatory constituent of *Urtica circularis*. J. Nat. Prod., 74: 1503-1507.

Masteikova, R., J. Bernatoniene & S. Velziene. 2008. Antiradical activities of the extract of *Passiflora incarnata*. Acta Pol. Pharm., 65: 577-583.

Megbowon, I. & T.O. Mojekwu. 2014. Tilapia sex reversal using methyl testosterone (MT) and its effect on fish, man, and environment. Biotechnology, 13: 213-216.

Mlalila, N., C. Mahika, L. Kalombo, H. Swai & A. Hilonga. 2015. Human food safety and environmental hazards associated with the use of methyl testosterone and other steroids in production of all-male tilapia. Environ. Sci. Pollut. Res., 22(7): 4922-4931.

Moon, Y.J., X. Wang & M.E. Morris. 2006. Dietary flavonoids: effects on xenobiotic and carcinogen metabolism. Toxicol. Vitro., 20: 187-210.

Obaroh, I.O. & G.C. Achionye-Nezh. 2013. Antifertility effect of some plant leaf extracts on the prolific breeding of *Oreochromis niloticus*. Asian J. Agric. Res., 2(12): 87-94.

Olthof, M.R., P.C. Hollman, P.L. Zock & M.B. Katan. 2001. Consumption of high doses of chlorogenic acid, present in coffee, or of black tea increases plasma total homocysteine concentrations in humans. Am. J. Clin. Nutr., 73: 532-538.

Peña-Mendoza, B., J. Gómez-Márquez & G. García-Alberto. 2011. Ciclo reproductor e histología de las gónadas de tilapia *Oreochromis niloticus* (Perciformes: Cichlidae). Cienc. Pesq., 19: 23-36.

Saarinen, N., S.C. Joshi, M. Ahotupa, X. Li, J. Ämmälä, S. Mäkelä & R. Santti. 2001. No evidence for the *in vivo* activity of aromatase-inhibiting flavonoids. J. Steroid Biochem., 78: 231-239.

Shu-Yao, Z., J. Hong, L. Chao-Xian, Z. Zhi-Wei, X. Dai-Shan & C. Lei. 2013. *In vitro* evidence of possible influence of scutellarein towards bile acids' metabolism. Afr. Health Sci., 13: 556-559.