THE EFFECT OF PAWPAW SEED MEAL AND MORINGA SEED MEAL ON OVARIAN DEVELOPMENT AND EGG VIABILITY IN SEXUALLY IMMATURE MOZAMBIQUE TILAPIA (*Oreochromis mossambicus*)

Ampofo-Yeboah, A [1*] and Owusu-Frimpong M. [2]

[1] *Department of Fisheries & Aquatic Resources Management, University for Development Studies, Nyankpala Campus, Box TL1882, Tamale*

[2] *Department of Applied Biology, Navrongo Campus, University for Development Studies, Tamale*

**Corresponding author: Akwasi Ampofo-Yeboah - ampoyeb@yahoo.com*

ABSTRACT

Tilapia culture efforts in Sub-Sahara African (SSA) countries are inefficient due to limited access to advanced biotechnologies to manage the precocious breeding strategy leading to overstocking in culture systems, which result in stunted growth and variable sizes of marketable fish. Developing suitable cost-efficient alternative methods to resource-poor communities in SSA to ensure sustainable tilapia production for food security purposes is warranted. A potential method to control the precocious breeding nature of tilapia is the use of natural plants components that contain endocrine disrupting compounds (EDCs). Several studies report on the antifertility effect of Pawpaw (Carica papaya) and Moringa (Moringa oleifera) seeds in certain livestock species. This study investigated the potential effect of Pawpaw (P) and Moringa (M) seed powders as phytogenics on ovary development in sexually immature Mozambique tilapia (Oreochromis mossambicus). The P and M seed powders were added to a commercial tilapia diet (NutroScience (Pty) Ltd, South Africa; the basal diet, BD) at inclusion levels of 0, 0.5, 1.0, 2.0, 5.0 and 10.0 g/kg BD, and fed to 1,650 sexually immature tilapia of unknown gender (2-8g; 30 fish per replicate; 5 replicates per treatment) for 60 days. After, 4 fish were sacrificed per replicate to assess the effects of P or M on ovary development. Fish fed 0g /kg BD, ovaries appeared normal, equal in size containing ochre coloured eggs. Egg production, ovulation and spawning were all affected, as evident in the difference in colour of the degrading eggs, as well as the absence of spawning. Eggs were observed in the ovaries of sexually immature fish, but spawning did not occur. Observations in fish fed diets included at 2.0, 5.0 and 10.0g P or M /kg BD showed deformities including unequal size, enlarged ovaries, twisted ovaries, a single and fused ovary. The study indicated that P and M dosage ≥2.0g/kg BD have a significant effect on normal ovary development and maturation in sexually immature female O. mossambicus. Thus, P or M seed powder can be used as potential EDCs to delay or suppress the attainment of sexual maturity in female tilapia, thereby, controlling tilapia precocious breeding. Sustainable availability of Pawpaw and Moringa seeds in SSA contributes to the attractiveness of their potential usage in the reproductive management of tilapia in commercial and small-scale tilapia culture systems.

KEYWORDS: *Oreochromis mossambicus*, Pawpaw, Moringa, ovarian development, tilapia, endocrine disruption

INTRODUCTION

Tilapias have become the most important group of aquaculture species, second to carp, with an annual world production of 2.5 million tonnes. Tilapia species gained popularity as a food species after their introduction to tropical, subtropical and temperate regions all over the world, being cultivated primarily for food, recreational use, aquatic weed control, and research purposes (Pilay, 1997; El-Sayed, 2006).

Tilapia culture in Sub-Sahara African (SSA) countries are hampered by limited access to advanced biotechnologies to manage the precocious breeding strategy of tilapia, which results in an overstocking of culture systems, with eventual stunted growth and variable sizes of marketable fish. Developing a simple and cost-effective method to control precocious breeding in tilapia may enable rural SSA communities to produce tilapia as cost-effectively as possible, thus contributing to food security.

A potential method to control the precocious breeding nature of tilapia is the use of natural plant components that contain endocrine disrupting compounds (EDCs). Previous studies have shown that phytochemicals (i.e. chemicals derived from a plant) occurring in medicinal plants have an estrogen-like biological activity (Turker and Takemura, 2011). Studies have shown that some phytochemicals are natural steroid-like substances with estrogen-like biological activity, and are, thus called phytoestrogens (i.e. estrogenic compounds found in plants). Phytoestrogens are described as naturally occurring plant compounds that are structurally and/or functionally similar to mammalian estrogens and their active metabolites, by mimicing and / or modulating the actions of endogenous estrogens (such as 17β-estradiol) usually by binding to estrogen receptors. These compounds have the capacity to alter the structure or function(s) of the endocrine system and cause adverse effects including; the timing of puberty, capacity to produce viable and fertile offspring, sex specific behaviour; premature reproductive senescence, and compromise fertility (Patisaul and Jefferson, 2010).

Any substance that can interfere with the activity of either estrogens or androgens, can lead to defect in the reproductive health of the fish. There are considerable evidence, which indicate that exposure of an organism to EDCs or natural hormones which interfere with the normal functioning of the endocrine system has the potential to affect the reproductive endocrine function, and change reproductive development (Jobling *et al.*, 1998; Damstra *et al.*, 2002; Manning, 2005; Mills and Chichester, 2005; Cheshenko *et al.*, 2008; Sassi-Messai *et al.*, 2009; Blazer *et al.*, 2012). The state of fish gonad and reproduction are regarded as the more credible or reliable indictors of endocrine disruption in aquatic systems by both natural and artificial chemicals (Jobling *et al.*, 1998; Celino *et al.*, 2009).

A primarily plant diet for example omnivorous tilapias in the wild, and the use of soybean in the diet of fish in captivity, may be a source of considerable amounts of phytoestrogens (Pelissero and Sumpter, 1992). The effect of phytoestrogens on fish were initially documented and / or studied in trout and sturgeon (Pelissero *et al.*, 1991; Pelissero and Sumpter, 1992; Pelissero *et al.*, 1996).

The reported phytoestrogens identified in Pawpaw plant parts include β-sitosterol, saponins, and flavonoids (Krishna *et al.*, 2008) and that of Moringa plant parts include flavonoids (quercetin and kaempferol), saponins, triterpenes, e.g. oleanolic acid-3-glucoside, and β-sitosterol (Anwar *et al.*, 2007; Kumar *et al.*, 2010; Kumar *et al.*, 2012). The two plants (Pawpaw and Moringa) abound in SSA and, are used in traditional medicine. Several studies report on the antifertility effect of Pawpaw (*Carica papaya*) and Moringa (*Moringa oleifera*) seeds in certain livestock species. They have been demonstrated to possess abortifacient and/or antifertility properties (Das, 1980; Udoh and Kehinde, 1999; Bose, 2007).

Thus, they can be exploited in the quest for a more reliable solution to tilapia precocious breeding, hence could encourage tilapia culture in rural SSA for poverty alleviation. This method of control if proven to be effective could be easier to adopt by poor fish farmers, particularly in SSA, since Pawpaw and Moringa seeds used in this study are available all year round in the tropics and subtropical regions.

The study, therefore, aimed to investigate the effect of Pawpaw and Moringa seeds powder on ovarian development in sexually immature Mozambique tilapia (*Oreochromis mossambicus*).

MATERIALS AND METHODS

Experimental Location and Facilities

The study was conducted in a recirculated aquaria system (RAS) based at the Welgevallen Experimental Farm, Stellenbosch, South Africa. Welgevallen Experimental Farm is located at 33°56' 33.95" S, and 18°51'56.15 "E. The experimental facilities consisted of a recirculating aquaria system that housed a total of 72 glass aquaria. The RAS consisted of two platforms; each platform had six racks, and each rack had two levels, with three tanks per level. Each tank had a volume of 121L (dimensions 57cm x 53cm x 40cm).

Experimental Fish and Plant Materials

A total of 1,650 sexually immature Mozambique tilapia (*Oreochromis mossambicus*) weighing between 2 and 8g, were obtained from the general stock maintained at the Welgevallen Experimental Farm. The fish were randomly allocated to 55 tanks, at a stocking density of 30 fish (with unknown sex) per tank. The fish were allowed to acclimatize for 7 days, and after this period, the treatment diets were fed for period of 60 days. The fish were fed *ad libitum* (Baker, 1984) at three intervals, i.e. 08:00-09:00, 12:00-13:00, and 16:00-17:00.

Each aquarium was cleaned daily throughout the duration of the study. The mechanical filter (a plastic basket filled with Japanese mat, aquastones and foam) was cleaned every second day by removing the packaging and washing off all the solid and trapped materials. The larger tank units were cleaned on a weekly basis, with 10% of the volume being replaced and 1 kg common salt added to the system.

Pawpaw (*Carica papaya* Linn.) and Moringa (*Moringa oleifera* Lam.) seeds were used as potential sources of phytochemicals, collected and processed into powder. The powder form was then included as a supplement in a commercially available tilapia diet, Aquanutro (Nutroscience (Pty) Ltd, South Africa), which was used as the control or basal diet.

Experimental Design

The experimental design was a 3x5 factorial, with the three treatments being the commercially formulated tilapia feed as control or basal diet (BD), and the remaining two treatments of Pawpaw seed meal (P) and Moringa seed meal (M). The experimental compounds were included at five different inclusion levels, i.e. 0, 0.5, 1.0, 2.0, 5.0, and 10.0 g/kg BD. The experimental diets were fed for a period of 60 days, after an acclimatization period of 7 days, to sexually immature Mozambique tilapia, weighing between 2 and 8g. At the end of the trial, 20 fish were sacrificed per treatment group to assess the effects of P or M on ovary development. The study was conducted between April 2011 and June 2011, and the experiment was repeated 5 times.

RESULTS

Ovaries of sexually immature Mozambique tilapia (*Oreochromis mossambicus)* that received diets containing different levels (0, 0.5, 1.0, 2.0, 5.0, & 10.0g/kg basal diet) of Pawpaw seed meal (P) or Moringa seed meal (M) as supplements to a commercial tilapia diet, over a period of 60 days are shown in figures 1(a-e). Ovaries of *O. mossambicus* obtained that received the control diet (0g/kg BD) appeared normal, equal in size, and contained ochre coloured eggs (Figure 1a).

Figure 1a. Normal ovaries, similar in size and shape of Oreochromis mossambicus fed 0 g/kg basal diet

Figure 1a. Normal ovaries, similar in size and shape of *Oreochromis mossambicus* fed 0 g/kg basal diet

Fish that received diets containing 2.0, 5.0 and 10.0g of P or M /kg BD, respectively, had deformed gonads, with deformities including ovaries of unequal size (Fig 1b), twisted ovaries (Fig 1c), enlarged ovaries (Fig 1d), and a single fused ovary (Fig 1e). The study indicated that an inclusion level of P and M equal to or higher than 2.0g/kg BD have a significant effect on normal ovary development and maturation in sexually immature female *O. mossambicus* (Fig 1 b – e).

Figure 1b. Unequal sized ovaries of O. mossambicus fed 2.0, 5.0 and 10.0 g/kg basal diet of both P and M

Figure 1c. Twisted ovaries of O. mossambicus fed 2.0, 5.0 and 10.0 g/kg basal diet of both P and M

Figure 1d. Enlarged ovaries with degenerated or deformed eggs (abnormal colour), fed 2.0, 5.0 and 10.0 g/kg basal diet of both P and M.

Fig1e. Single fused ovary of Oreochromis mossambicus fed 2.0, 5.0 and 10.0 g/kg basal diet of both P and M.

DISCUSSION

The gonads of a sexually maturing Mozambique tilapia (*Oreochromis mossambicus*) resembles that of a gonochoristict teleost fish, where the female gonad consist of two distinct ovaries of similar size, which are joined at their posterior ends, and open through a common oviduct in the urogenital papilla (Yamazaki, 1983; Morrison *et al.*, 2006). In this study, normal ovaries were observed in fish receiving the control basal diet (BD) (Fig1a). When the gonadal development of fish sampled from the respective treatment groups are compared to that of the control (Fig 1a), deformed ovaries were observed in fish fed with diets containing 2.0, 5.0 and 10.0g/kg basal diet of Pawpaw (P) or Moringa (M) (Fig1 b-e). The most prominent deformities included ovaries that were unequal size (Fig 1b), and ovaries twisted at the mid-section (Fig 1c), Other visible deformities included enlarged ovaries filled with a large number of white to cream-coloured eggs (Fig1d) and a single or fused ovary (1e). The majority of the females who carried deformed ovaries, had distended abdomens, which could indicate a disturbance of endocrine disruption in the fish (Jobling *et al.,* 1998; Celino *et al.,* 2009). The eggs in the ovarian cavity will degenerate and reabsorbed (Fig 1d), in such situations could cause the death of the fish (Mañanos *et al.,* 2009).

The appearance of eggs observed in females from the basal diet (Fig 1a) conformed to what has been described for the *Oreochromis* species, i.e. as having an oval shape, being relatively large, and being yolky with yellowish to ochre colouration or orange-yellow in colour (Rana, 1988; MacIntosh and Little, 1995; Morrison *et al.*, 2006). *Oreochromis* species cultured in aquaculture production systems are reported to normally produce eggs that are ochre (i.e. red to yellow earth) in colour. The intensity of colour, however, is influenced largely by nutrition, for example, the quantity of pigments such as carotenoids in the diet of brood fish (Rana, 1988). *Oreochromis mossambicus* produces eggs that are yellow in colour (Shelton and Popma, 2006). When the ova of fish in this study are considered, they contained eggs that were white to cream in colour (Fig 1b-e). This is potentially an indication that the eggs were abnormally formed. This is supported by the fact that although eggs were observed in the ovaries of the fish that were samples in this study, no spawning and/or females brooding eggs were observed for the treatment groups receiving 2.0, 5.0 and 10.0 g/kg basal diet of both P and M.

Oocyte enlargement can be ascribed to the accumulation of yolk, formed from vitellogenin (a female specific protein), which is synthesized by liver in response to 17β-estradiol, released into the blood stream, and later transported to the ovary (Nagahama, 1983; Tyler and Sumpter, 1996). The phytochemicals present in the Pawpaw and Moringa potentially mimicked the biological effect of endogenous hormones such as 17β-estradiol, preventing the processing and incorporation of vitellogen, resulting in liquefaction or vacuoles forming in the eggs, reducing the viability of the eggs.

Atresia a process of follicle degeneration, or the resorption of developing oocytes could occur if estrogens which are indispensable for the growth and maturation of follicles activities are curtailed. Atretic follicles exhibit decreased estrogen production and a lower estrogen/androgen ratio in the follicular fluid, suggesting the importance of local estrogens for the maintenance of healthy follicles (Mañanos *et al.,* 2009). Atretic follicles are also characterised by reduced aromatase activity, leading to decreases of estrogen production and accumulation of androgen (Blazer, 2002; Mañanós *et al.*, 2009). The observed enlarged gonads could occur possibly due to the phytoestrogens from the Pawpaw and Moringa antagonizing the effects of estrogens by binding to the receptor but not activate and / or alter the pattern of synthesis and metabolism of it. In this case, the presence of the chemical on the receptor prevented the binding of the natural hormone preventing the final maturation and ovulation. Thus, instead of the fish ovulating and spawning the eggs stays in the ovary leading to its enlargement. From the above explanation it is clear that the enlarged ovaries could not have been follicular atresia, but different process did occurred to elicit the observed effect in the fish.

The female fish in captivity experiences reproductive dysfunction, caused by stress and absence of environmental cues (i.e. light and temperature) for reproduction. Studies have shown that three forms of reproductive dysfunction that could affect egg quality involves inhibition of vitellogenesis, inhibition of the final oocyte maturation and inhibition of the process of spawning (Mañanós *et al.*, 2009). According to Pavlov *et al.* (2009), the quality of an egg depends on its inherent properties and the environment in which the egg develops from fertilization. The egg's ability to produce a viable offspring depends on the genotype, as well as the morphological, biochemical and physiological processes happening inside the egg. Furthermore, poor egg quality (i.e. spoilt eggs) can be caused by the dysynchronization in the processes of egg maturation and ovulation which could bring about under-ripening or over-ripening and disturbances in egg maturation (Mañanós *et al.*, 2009; Pavlov *et al.*, 2009). Therefore, the spoilt ova which were encountered, might have been caused by dysynchronization of egg maturation.

It has been established that, in teleost fish at the end of gametogenesis pituitary Luteinizing-Hormone (LH) secretion induces the synthesis of maturation-inducing steroids (MIS), which regulate the process of gonadal maturation. After maturation is completed, a Gonadotropin Releasing Hormone (GnRH) induced LH surge stimulates ovulation. Some of the reported anomalies attributed to endocrine disrupting compounds in aquatic environments include morphological deformation of the gonads and asymmetrical development (Jobling *et al.*, 1998). One major reproductive dysfunction of females is inhibition of spawning which prevents the eggs from shedding out of the oviduct thereby the ovulated eggs remain in the ovarian cavity (Mañanós *et al.*, 2009; Pavlov *et al.*, 2009). Therefore, the deformed gonads and spoilt eggs in this study were as results of the presence of the phytoestrogens present in the Pawpaw and Moringa seeds. This could account for the enlargement of the ovary with a huge volume of over-ripening eggs (Fig 1d).

Egg production, ovulation and spawning were all affected, as evident in the difference in colour of the degrading eggs, as well as the absence of spawning. Eggs were observed in the ovaries of sexually immature fish, but spawning did not occur.

This clearly indicates that Pawpaw and Moringa possess compound or chemicals that affect the integrity of gonads and eggs; therefore, they can be used to manipulate reproduction in developing or maturing tilapia to prevent it spawning in aquacultural system.

CONCLUSION

The inclusion of Pawpaw (P) or Moringa (M) seed powder at levels equal or higher than 2.0g/kg BD proved to be successful in delaying or suppressing ovarian development in sexually immature female tilapia. Pawpaw seed powder and Moringa seed powder are potential endocrine disrupters which could be exploited in delaying puberty in tilapia. Future studies should investigate the influence of the EDCs present in Pawpaw and Moringa seeds on egg formation, maturation and eventual release. Sustainable availability of Pawpaw and Moringa seeds in Sub-Saharan Africa contributes to the attractiveness of their exploitation in the reproductive management of tilapia small-scale tilapia culture systems.

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