ผลของอาหารผสมฮอร์โมนพรอสตาแกลนดิน เอฟ ทู แอลฟา ต่อประสิทธิภาพการเจริญเติบโตและการสืบพันธุ์ของปลานิลเพศเมียในกระชัง

Effect of dietary prostaglandin F_2 administration on growth and reproductive performance of female Nile tilapia *Oreochromis niloticus* in cage culture

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บทคัดย่อ

การศึกษาผลของฮอร์โมนพรอสตาแกลนดิน เอฟ ทู แอลฟา ความเข้มข้นต่างกันต่อประสิทธิภาพการเจริญเติบโตและการสืบพันธุ์ของปลานิลเพศเมีย น้ำหนักเฉลี่ยเริ่มต้น เท่ากับ 182.03±1.93, 181.50±1.41, 181.10±1.58 และ 181.55±1.59 กรัมต่อตัว สำหรับกลุ่มที่ 1, 2, 3 และ 4 ตามลำดับ โดยเลี้ยงปลาทดลองในกระชัง ขนาด 3×3×1 เมตร แขวนลอยบนบ่อดิน โดยไม่มีการควบคุมสภาพแวดล้อมการทดลอง ปล่อยปลาที่ความหนาแน่น 4 ตัว/ตรม. ตลอดระยะเวลาการทดลอง 60 วัน ให้ปลากินอาหารเม็ดสำเร็จรูป (ระดับโปรตีน 30 เปอร์เซ็นต์) เคลือบด้วยสารละลายฮอร์โมน $PGF_{2\alpha}$ สังเคราะห์ ความเข้มข้นเท่ากับ 200, 500 และ 1,000 ไมโครกรัมต่ออาหาร 1 กิโลกรัม สำหรับกลุ่มที่ 2, 3 และ 4 ตามลำดับ) ส่วนทดลองที่ 1 ให้ปลากินอาหารเม็ดสำเร็จรูปอย่างเดียว ผลการศึกษาพบว่าประสิทธิภาพการเจริญเติบโตของปลาทดลองทั้ง 4 กลุ่ม ไม่มีความแตกต่าง ($P > 0.05$) ในขณะที่ผลการศึกษาด้านประสิทธิภาพการสืบพันธุ์พบว่า ปลาทดลองกลุ่มที่ 4 มีค่าปริมาณไข่ทั้งหมดและอัตรารอดตายของลูกพันธุ์ปลาสูงสุดอย่างมีนัยสำคัญทางสถิติ ($P < 0.05$) เมื่อเปรียบเทียบกับกลุ่มทดลองที่1, 2 และ 3 ตามลำดับ สรุปผลการศึกษาข้างต้นชี้ให้เห็นว่าการให้แม่พันธุ์ปลานิลกินฮอร์โมน $PGF_{2\alpha}$ ผสมอาหาร ไม่มีอิทธิพลต่อประสิทธิภาพการเจริญเติบโตแต่ส่งผลต่อการเพิ่มปริมาณไข่และอัตรารอดตายของลูกพันธุ์ปลา โดยองค์ความรู้ที่ได้จากการศึกษาครั้งนี้จะเป็นประโยชน์สำหรับการสร้างอาหารผสมฮอร์โมนควบคุมประสิทธิภาพการสืบพันธุ์ของแม่ปลานิล

คำสำคัญ: พรอสตาแกลนดิน เอฟ ทู แอลฟา ปลานิล ประสิทธิภาพการเจริญเติบโตและการสืบพันธุ์

Abstract

The study on the effects of feeding female Nile tilapia with different levels of prostaglandin $F_{2\alpha}$ hormone ($PGF_{2\alpha}$) on growth and reproductive performance of fish were investigated in cage culture environments. Fish (182.03±1.93 (Group1), 181.50±1.41 (Group2), 181.10±1.58 (Group3), and 181.55±1.59 (Group 4), $gfish^{-1}$) were used and cultured in cage (3x3x1 m) in the reservoir without controlling external factors of water at stocking density of 4 $fishm^{-2}$. During study, all fish were fed with commercial fish diet (%30CP) at 3% $g.bw^{-1}$for 60 days. Then, they were divided in to four treatments group with different level of $PGF_{2\alpha}$ hormone supplement at 200, 500 and 1000 $\mu gPGF_{2\alpha}$ kg^{-1}diet for group 2, 3 and 4, respectively. While fish in group1 was fed only commercial fish diet without $PGF_{2\alpha}$ hormone supplement. The result showed that the data of growth performance of female fish in all groups of study were no significant

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differences (*P > 0.05*). While, the data of reproductive performance especially total egg production and percentages larvae survival rate of Nile tilapia fish in group4 was significantly different (*P<0.05*) among treatment groups. In conclusion, it was clearly shown that dietary supplementation of $PGF_{2\alpha}$ hormone had no significant influence on growth performance of female fish, but were significantly different (*P < 0.05*) on total egg production and percentage of larvae survival rate of female Nile tilapia. This knowledge can be used information for establish the hormone diets for controlling populations of female Nile tilapia fish.

Keywords: Prostaglandin $F_{2\alpha}$, *Oreochromis niloticus*, growth and reproductive performance

Introduction

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (fig.1) is a group of cell signaling molecules in the same eicosanoid family[1]. It is synthesized enzymatically from one of the three highly unsaturated 20-carbon fatty acids directly from endoperoxide PGH_2 and the action of $PGF_{2\alpha}$ is dependent on the number of receptors on the target cell membrane[2]. $PGF_{2\alpha}$ is rapidly metabolized to 13,14-dihydro-15-keto $PGF_{2\alpha}$ in vivo, and is an important bioactive mediator for many physiological functions in animals[3]. Similar results have been reported by Adams et al.[4] who found that $PGF_{2\alpha}$ stimulates protein synthesis of skeletal and smooth muscle cells in culture and is elevated in the heart during compensatory growth of neonatal rat cardiac myocytes. In addition, Horsley and Pavlath[5] demonstrated that $PGF_{2\alpha}$ as well as two analogues augment muscle cell size in vitro. Increased myotube size was not due to $PGF_{2\alpha}$-enhancing cell fusion that initially forms myotubes, but rather to $PGF_{2\alpha}$ recruiting the fusion of cells with preexisting multinucleated cells. In vertebrates and invertebrates, the functions $PGF_{2\alpha}$ have been reported. $PGF_{2\alpha}$ increase during the final stages of gonadal development; also it acts not only as a female reproductivehormone, but also as a sex pheromone influencing fish reproductive behaviors[6]. In most species of teleost fishes, and in contrast with other vertebrates, $PGF_{2\alpha}$ have been reported to have functional abilities that stimulate sexual displays in regulation of sexual behaviour in female guppy, *Poecilia reticulata*[7]. Earlier work Lister and Van Der Kraak[8] has suggested that naturally spawning groups of female zebrafish exhibit increased ovarian levels of $PGF_{2\alpha}$, and 17_{α}, 20 β-dihydroxy-4-pregnen-3-one (a maturation-inducing hormone in zebrafish) near the time of ovulation compared with non-breeding females. Similarly, the study of arachidonic acid induced production of 17_{α}, 20 β -dihydroxy-4-pregnen-3-one (DHP) via a putative PGE_2 receptor in fish follicles from the Eurasian perch found that arachidonic acid and its derivatives, PGE_2 and $PGF_{2\alpha}$, act on final follicle maturation.[9] Another study of the effect of $PGF_{2\alpha}$ on goldfish showed that factors associated with ovarian development have only minor influence at most on $PGF_{2\alpha}$ induced female spawning behavior[10]. Recently, the number of studies identifying the function of $PGF_{2\alpha}$ in aquatic animals has been increasing in response to the commercial value of many aquatic animals and the action of $PGF_{2\alpha}$ in promoting reproductive performance[7-10], but influence on growth performance has not yet been documented. Hence, the purpose of this study was to further evaluate the effect of $PGF_{2\alpha}$ administration on the growth and reproductive performance of female Nile tilapia brooders in cage culture. Knowledge on the role of $PGF_{2\alpha}$ on the growth and reproductive performance of Nile tilapia fish and the changes of water quality could be used to improve the production of gametes and larvae of this fish, that is an important commercial aquaculture species in the tropical areas, including Thailand.

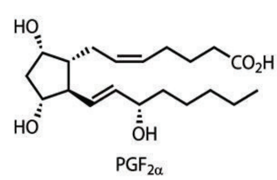

Figure.1 Structure of Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)[11]

Materials and methods

Experimental Feeds

The commercial fish diet that used in this study were different crude protein (CP), 30% CP, and it were selected based on the essential nutrients necessary for a satisfactory growth rate of female Nile tilapia fish. Four experimental diets were formulated with different synthetic $PGF_{2\alpha}$ hormone (Cayman Chemical, Thailand) supplement. Commercial fish diet enriched with synthetic $PGF_{2\alpha}$ hormone solution with the dosage of 0, 200, 500 and 1000 µg$PGF_{2\alpha}$ kg^{-1}diets for Group1, Group2, Group3, and Group4, respectively and stored in airtight polyethylene plastic bags at 4 °C until used.

Experimental design

The study consisted of one experiment with Completely Randomize Design in double replicates per group. , Three hundred of Nile tilapia fish brooders (181 g.bw, live body weight of 5 months olds approximately and an average total length of 22 cm) were selected for use in this experiment. Fish were collected during April, 2015 from a commercial freshwater fish farm in Tak province. Before the experiment, fish were reared in two tons in fish tank for adoption with freshwater over 7 days and dramatically substituted with commercial fish diet until the fish accepted the experimental diets. At the beginning of culturing, a random sample of experimental fish was individually weighed and released in each treatment group, 9 m^3, 3x3x1 m cage culture without aeration. The stocking density of fish was 4 fish.m^{-2} in the cage and the ratio of female:male was 1:1. During the experimental period, from May to June, 2015, the fish were fed with each experimental diet until satiation, about 3% g.bw^{-1} one time a day at 09:00 am. During the experimental period in the reservoir, the water temperatures were observed two times every day in the morning and afternoon. The minimum and maximum water temperature in the morning ranged from 26 to 27 °C and 29.50 to 30 °C, respectively. During the experimental period, dissolved oxygen (DO), pH and turbidity were monitored every week. In the reservoir, DO, pH and turbidity ranged from 5.23 to 7.10 mgL^{-1}, 7.07 to 7.70 and 0.50 to 1.19 NTU, respectively. Ionized ammonia (NH_4^+) nitrite (NO_2^-) and nitrate (NO_3) concentration of water in the reservoir were biweekly observed using a water test kit, KYORITSU CHEMICAL-CHECK Lab., Corp., Japan. Ionized ammonia (NH_4^+) nitrite (NO_2^-) and nitrate (NO_3) in the reservoir during experiment ranged from 0.03 to 0.13 mgL^{-1}, 0.03 to 0.13 mgL^{-1} and 0.01 to 0.08 mgL^{-1}, respectively.

Data collection and analytical methods

Growth performance

At the end of culturing, growth performance of fish measured as mean final weight, percentage of weight gain, average daily gain and specific growth rate were determined. Fish fed diets containing 0, 200, 500 and 1000 µg$PGF_{2\alpha}$ kg^{-1}diet were evaluated by weighting all of the population. This study was conducted for 60 days in cage culture in the reservoir (3 Rai). The following indices were used to evaluate the fish growth performance according to the method described by Olvera-Novoa et al.[12] as follows: Mean final weight (g $fish^{-1}$) = [(Mean final fish weight – Mean initial fish weight)]/Culture period (day), Percentage of weight gain (%) = [(Mean final fish weight – Mean initial fish weight) X100]/ Mean initial fish weight, Specific growth rate, (% day^{-1}) = [(ln final fish weight – ln initial fish weight) X 100]/ Culture period (day)

Reproductive performance

Every week, the eggs were collected from the oral cavity of individual female fish in each treatment group by counter-flow of the oropharynx. The weight of experimental fish was recorded. The eggs were transferred indoors for hatching in incubators (10 liter capacity in each unit) at a stocking level of 200 eggs per liter. A steady current of freshwater at 5 liter min^{-1} was allowed to pass through each incubator after being mixed with oxygen as soon as it was out of the bio-filter tank. Everyday, hatching and non-hatching eggs were recorded while larval survival rate was recorded after 10 days of larvae hatching, from which the reproductive performance were evaluated. The following indices were used to evaluate reproductive performance according to the method described by Almeida et al.[13] as follows: Total egg production (eggs g.bw^{-1}) = [Total number of eggs produced during culture period]/Total final fish weight (g), Percentages of fertilization rate (%) = [Total number of fertilized eggs during culture period X 100]/Total number of eggs during culture period, Percentages hatching rate (%) = [Total number of larvae during culture period X 100]/ Total number of eggs during culture period, Percentages larvae survival rate (%) = [Total number of larvae after 10 days of hatching X 100]/Total number of larvae during culture period.

Statistical analyses

All data was expressed as mean±SD and analyzed by one-way ANOVA. The Duncan's Multiple Range Test at $P < 0.05$ was used to determine the differences between the groups means[14].

Results

The study on the effects of feeding female Nile tilapia with different levels of prostaglandin $F_{2\alpha}$ hormone on growth and reproductive performance of fish was conducted for 60 days. The results of this study are presented below:

Growth performance

According to Table1, initial fish body weights were 182.03±1.93, 181.50±1.41, 181.10±1.58, and 181.55±1.59 g of Group1, Group2, Group3 and Group4, respectively. The final fish body weight at the end of the experimental period (60 days) were not significantly different among treatment groups and were highest on fish Group4 (223.20± 5.57 g) followed by Group2 (220.40±4.93 g) Group 3 (219.70±3.71 g) and Group1 (219.10±3.25 g), respectively. Mean final weight ($gfish^{-1}$) at the end of the experimental period (60 days) were highest on fish in group4 (0.69±0.10 $gfish^{-1}$) followed by Group2 (0.65±0.08 $gfish^{-1}$) Group3 (0.64±0.06 $gfish^{-1}$) and Group1 (0.62±0.06 $gfish^{-1}$), respectively. Percentage of weight gain at the end of the experimental period were highest on fish in Group4 (22.96±3.53%) followed by Group2 (21.44±2.69%) Group 3 (21.32± 2.05%) and Group1 (20.38±2.16%). Specific growth rate at the end of the experimental period (60 days) were highest on fish in Group4 (0.34±0.05 $\%day^{-1}$) followed by Group2 (0.32±0.04 $\%day^{-1}$) Group3 (0.32±0.03 $\%day^{-1}$) and Group1 (0.31±0.03 $\%day^{-1}$). However, three parameters of growth performance, namely mean final weight, percentage of weight gain, and Specific growth rate, there were not significantly different ($P>0.05$) among treatment groups

Table 1 Growth performance of female Nile tilapia with different levels of $PGF_{2\alpha}$ during the 60 days of experiment (Mean±SE).

Growth performance	Group1	Group2	Group3	Group4	F-Value	Sig.
Mean initial weight (g)	$182.03±1.93^{a}$	$181.50±1.41^{a}$	$181.10±1.58^{a}$	$181.55±1.59^{a}$	0.54	0.65
Mean final weight (g)	$219.10±3.25^{b}$	$220.40±4.93^{ab}$	$219.70±3.71^{ab}$	$223.20±5.57^{a}$	1.65	0.20
Mean weight gain (g)	$0.62±0.06^{b}$	$0.65±0.08^{ab}$	$0.64±0.06^{ab}$	$0.69±0.10^{a}$	1.52	0.23
Percentage of weight gain (%)	$20.38±2.16^{b}$	$21.44±2.69^{ab}$	$21.32±2.05^{ab}$	$22.96±3.53^{a}$	1.60	0.21
Specific growth rate ($\%day^{-1}$)	$0.31±0.03^{b}$	$0.32±0.04^{ab}$	$0.32±0.03^{ab}$	$0.34±0.05^{a}$	1.40	0.26

Note: Means with different superscript (a, b) in the same row were significantly different ($P<0.05$).

Reproductive performance

According to Fig. 2, total egg production of fish given different concentration of $PGF_{2\alpha}$ hormones diets during 60 days showed significant differences among the treatment groups. Total egg production of fish fed diets containing 1000 $\mu gPGF_{2\alpha}kg^{-1}$diet was found to be the highest (1271.00±102. 33 eggs $g.bw^{-1}$) but the lowest (1107.00±142.21 eggs $g.bw^{-1}$) was in fish fed diets containing 0 $\mu gPGF_{2\alpha}$ kg^{-1}diet. However, the volume of total egg production of experimental fish fed with different concentration of $PGF_{2\alpha}$ diets tended to increase with increasing $PGF_{2\alpha}$ hormones level in the diets. Percentages of fertilization rate in fish fed diets containing different $PGF_{2\alpha}$ levels during 60 days showed no significant differences among fish fed diets containing 0, 200, 500 and 1000 µg$PGF_{2\alpha}$ kg^{-1}diet. They were highest in fish fed diets containing 1000 µg$PGF_{2\alpha}$ kg^{-1}diet (76.40±7.76%) but lowest in fish fed diets containing 200 µg$PGF_{2\alpha}$ kg^{-1} diet (67.80±9.16%). In the case of experimental fish fed diets containing 0 (Group1) and 500 (Group3) µg$PGF_{2\alpha}$ kg^{-1}diet, percentages of fertilization rate were 72.58±5.30 and 72.94±17.52, respectively. Percentages of hatching rate were not significantly different between group of 0, 200, 500 and 1000 µg$PGF_{2\alpha}$ kg^{-1}diet. Percentages of hatching rate in experimental fish fed diets containing different $PGF_{2\alpha}$ levels increased by increasing $PGF_{2\alpha}$ levels in the diets. Percentages of hatching rate were 70.90±5.64, 66.08±9.44, 69.72±14.87 and 71.62 ±8.72 of diets containing 0, 200, 500 and 1000 µg$PGF_{2\alpha}$ kg^{-1}diet, respectively. Percentage of larvae survival rate was significantly different among treatment groups and was highest on fish fed diets containing 1000 µg$PGF_{2\alpha}$ kg^{-1} diet (80.50 ±8.72) followed by fish fed diets containing 500, 200 and 0 µg$PGF_{2\alpha}$ kg^{-1}diet, respectively. Total egg production and percentage of larvae survival rate of female Nile tilapia with different levels of $PGF_{2\alpha}$ hormone during the 60 days of experiment is presented in Figure.2.

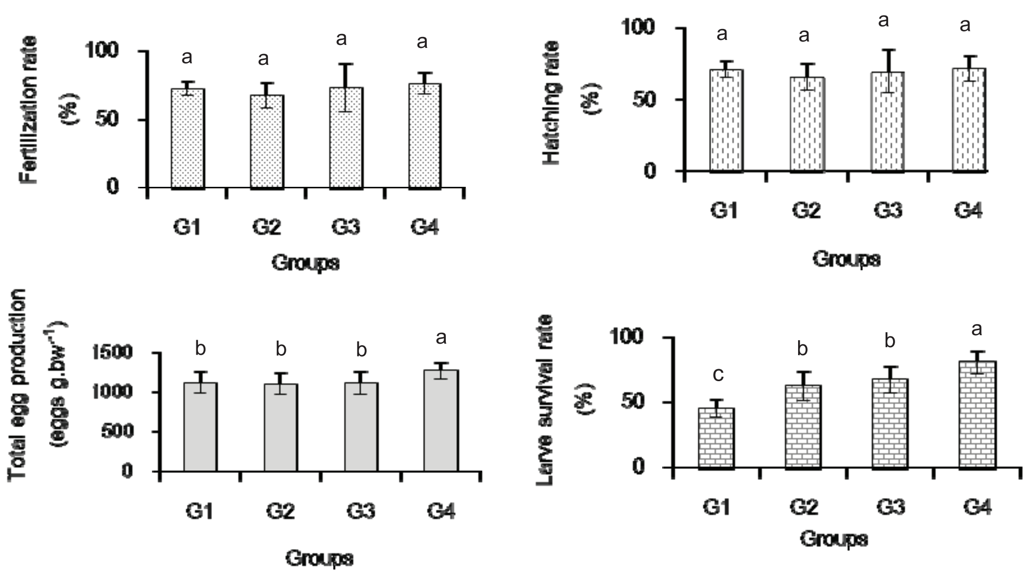

Figure 2 Hatching rate (%), fertilization rate (%), total egg production (eggs $g.bw^{-1}$) and larvae survival rate (%) of female Nile tilapia with different levels of $PGF_{2\alpha}$ during the 60 days of experiment (Means±SD, n=10 fish) represented in group1 (G1), group2(G2), group3(G3) and group4(G4) respectively. Significant differences ($P< 0.05$) in the value of some parameters of reproductive performance of experimental fish between treatment groups (G2, G3, and G4) and G1 are indicated with letters (a, b, and c).

Discussion

Studies using dietary $PGF_{2\alpha}$ in female Nile tilapia fish diets were conducted. Based on the statistical analysis, the results obtained from experiments showed that the growth responses of fish fed with diets contained 0, 200, 500 and 1000 µg$PGF_{2\alpha}$ kg^{-1}diet in terms of mean final weight, mean weight gain (g), percentage of weight gain and specific growth rate were increased by increasing $PGF_{2\alpha}$ levels in the experimental diets up to 1000 µg$PGF_{2\alpha}$ kg^{-1}diet. In these experiments, fish in Group4 (1000 µg$PGF_{2\alpha}$ kg^{-1}diet) showed higher mean final weight, mean weight gain, percentage of weight gain, and specific growth rate but were not significantly different ($P>0.05$) among treatment groups followed by fish in

Group3 Group2 and Group1, respectively. It is thought that $PGF_{2\alpha}$ may have a potential capacity to enhanced growth performance of female Nile tilapia fish, a conclusion supported by Denning-Kendall and Wathes[15] who reported that several studies indicate that prostaglandins are widely distributed in various tissues in animals and plants, which play an important role in growth and reproductive physiology, especially during ovarian steroidogenesis and metamorphosis. Similarly, studies on PGE2 induced growth hormone release and effects of intrahypothalamic and intrapituitary implants by Ojeda et al.[16] suggested that PGE2 can act at both hypothalamic (ARH-ME) and pituitary levels to stimulate GH release. At the hypothalamus, PGE2 may inhibit GH-inhibiting factor release or induce release of GH releasing factor. Furthermore, Stacey and Gqetz[17] mentioned that several studies indicate that prostaglandin E_2 and $PGF_{2\alpha}$, released from the ovaries or oviduct in response to the presence of ovulated oocytes also act on the brain to stimulate female spawning behavior in several spices of fish. Based on the statistical analysis, however, the increase in fish growth with increasing concentration of $PGF_{2\alpha}$ in the diets may have been caused by the essential component in the fish diet. In fish nutrition, the ratio between omega-3 and omega-6 poly-unsaturated fatty acids influences skeletal development. Supplementation of fish oils with vegetable oils increases the content of omega-6 fatty acids, such as arachidonic acid which are metabolized by cyclooxygenases to prostaglandin E2, an eicosanoid with effects on bone formation and remodeling[18]. The values of total egg production, fertilization rate and hatching rate were the highest at fish in Group4 and were significantly different (P<0.05) among treatment groups. It means that $PGF_{2\alpha}$ have a potential capacity to promote reproductive performance of female Nile tilapia fish. This agrees with Skoblina and Minin[19] who demonstrated that zebrafish oocytes that have undergone maturation under the indicated conditions ovulate when treated with prostaglandin$F_{2\alpha}$ (5 µg$PGF_{2\alpha}$ mL^{-1}) and/or 20% carp ovarial fluid and are capable of development towards the actively feeding larvae upon fertilization. Similarly, Laberge and Hara[20] research on behavioral and electrophysiological responses to F-prostaglandins, putative spawning pheromones, in three salmonid fishes include brown trout, lake white fish and rainbow trout. The result found that the behavioral and olfactory responses observed with exposure to $PGF_{2\alpha}$ and its metabolites suggested these compounds function as reproductive pheromones in brown trout and lake whitefish. Moreover, Kobayashi et al.[21] mentioned that in freshwater fish, the female goldfish, $PGF_{2\alpha}$ and its metabolites are released as a postovulatory pheromone that induces male spawning behavior which further increases male LH and sperm production. In invertebrates, too PGs have been reported to induce spawning in the abalone *Haliotis refescens*. Recently, several researchers such as Spaziani et al.[22] reported that PGs are related to vitellogenesis and spawning in aquatic animals. According to the results of the present study, the percentage of larvae survival rate was significantly different (P<0.05) among treatment groups and was highest in fish in Group4 and lowest on fish in Group1. It means that $PGF_{2\alpha}$ have a potential capacity to enhanced oocyte development of female Nile tilapia fish. These results agreed with some literature reports that $PGF_{2\alpha}$ encourages gonadal development in aquatic animals, with direct effects on embryo health and larval survival rate[3]. Based on statistical analysis, the results obtained from experiments showed that during 60 days of culture period, Nile tilapia fish were almost survived. This indicated that survivals were not affected by dietary $PGF_{2\alpha}$. Wedemeyer[23] mentioned that water quality was widely acknowledged to be one of the most important rearing conditions. The proper temperature for Nile tilapia is 25–30 °C, DO 0.3–0.6 ppm, and pH 6.5–9.0[24].

Conclusion

Based on the results gathered from the experiments, the following can be concluded:- The results showed that dietary supplementation of $PGF_{2\alpha}$ hormone had no significant influence on mean final weight, mean weight gain, percentage of weight gain, and specific growth rate of fish but they were higher in Nile tilapia fish in Group4 followed by fish in Group3 Group2 and Group1, respectively. However, dietary supplementation of $PGF_{2\alpha}$

had significant influence (*P<0.05*) on total egg production and percentage of larvae survival rate of female Nile tilapia in cage culture environments. Therefore, the level of 1000 µgPGF$_{2\alpha}$ kg^{-1}diet supplementation in fish diets was recommended for female Nile tilapia brooders in cage culture, when focus on total egg production and percentage of larvae survival rate of female Nile tilapia in cage culture.

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