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Original Article

Induced spawning of African catfish (*Clarias gariepinus*) using goat (*Capra aegagrushircus*) and pig (*Sus scrofa domesticus*) pituitary extracts

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Abstract
Concerns relating to cost and availability have directed traditional farmers to expand options on the induced spawning of fish for viable production of seeds. The study was conducted to examine the potential of using pituitary extracts (PE) from goat (*Capra aegagrushircus*) and pig (*Sus scrofa domesticus*) on the induced spawning of African catfish (*Clarias gariepinus*). Pituitary extract of African catfish using 4 mg kg^{-1} body weight dosage (control) and PE from goat and pig using 4 mg kg^{-1} and 200 mg kg^{-1} body weight dosages were single intramuscularly injected to female breeders. Utilisation of goat and pig PE has successfully induced the spawning of African catfish. All female breeders attained ovulation within 10-12 h which is under the prescribed latency period. Total number of eggs spawned ranged from 36200 to 67600. The three PE used attained comparable result in terms of relative fecundity. Fertilisation rate revealed that PE from goat at 4 mg kg^{-1} dosage attained similar result to that of control treatment. Hatching period occurred within 24 – 36 h and goat PE using both dosages gave the highest results in hatching rate. The current study provides preliminary information on the success of using PE from goat and pig for the induced spawning of African catfish.

Keywords: artificial spawning; fish hatchery; hormone dosage; mammalian pituitary extract; pituitary gland

1 | INTRODUCTION

The African catfish, *Clarias gariepinus*, is one of the most important aquaculture fish species because of its hardiness, ability to survive hypoxic condition, ability to accept pelleted feeds and fast growth in captivity (Ekunwe and Emokaro 2009). It is also an important aquaculture food that fetches a high market value (Oladosu *et al.* 1993; Ayinla *et al.* 1994). In 2015, the total production of African catfish was 246476 tonnes (FAO 2017). Nigeria is the top African catfish producing country followed by the Netherlands, Brazil, Hungary, Kenya, Syrian Arab Republic, South African, Cameroon and Mali (FAO 2016). In nature, African catfish has a discontinuous annual reproductive cycle with alternate periods of resting, pre-spawning and breeding, regulated by cyclically active gonadotrophes (van Oordt and Goos 1987). This fish does not spawn year-round and they only spawn once a year. The spawning season of African catfish in the countries of

North and South of the equator lasts from June until September and from November until February respectively (Richter 1979).

Aquaculture that is dependent on wild-bred fish seeds is fraught with several disadvantages such as inadequate supply of fish seed required to meet the production target of the farmer (Rottmann *et al.* 1991). Seed availability which is hindered by the scarcity of natural spawning in captivity and shortage of high quality fingerlings is the bottleneck for successful culture of farmed fish and the dependence on natural resources for seed collection is seasonal reliant, restricted, unreliable, time-consuming and uneconomic (El-Hawarry *et al.* 2016). To guarantee adequate supply of fingerlings with known age and genetic background, several studies have been carried out, which recent findings have led to improved techniques in induced spawning of African catfish (Viveen *et al.* 1986; Nwokoye *et al.* 2007; Akinwande *et al.* 2009; Ataguba *et al.* 2009). Induced spawning is a technique to stimulate ripe fish breeders by pituitary hormone or any other synthetic hormone to breed in captive condition by promotion of timely release of sperms and eggs (Rottmann *et al.* 1991). Administration of fish pituitary extract to induce breeding in fish has been a common standard practice in aquaculture (Halder *et al.* 1991). Pituitary gland is the main source of the major hormones responsible for reproduction in catfish and other farmed species. The hormonal induction involves the injection of pituitary gland extract from the donor fish of equivalent weight or from other species to the female spawned (Fagbenro *et al.* 1998; Salami *et al.* 2006).

The efficiency of different hormones including common carp pituitary extract (CPE), catfish pituitary extract, luteinizing hormone releasing hormone analog (LHRHa) and human chorionic gonadotropin (HCG) have been investigated for induced spawning (Su *et al.* 2013). However, scarcity of commercial hormones and pituitary extracts is one of the constraints in developing countries because these resources are not available locally and, in many cases, needed to be imported which projects additional expense in the cost that makes it more uneconomical. This urged traditional aquaculture farmers and hatcheries to look for other alternatives that can provide advantage in terms of practicality and can be generated locally (Olaleye 2005; Muchlisin *et al.* 2014). Others have reported the potency of pituitary extract of non-piscine extract from chicken (Andalusia *et al.* 2008), cattle (Oka 2005) and frog (Nwadukwe 1993) but no previous studies demonstrated the efficacy of using pituitary extracts from goat and pig. The result from this study is envisioned to provide additional options among farmers on the materials to use for better results in the induced spawning of African catfish. In this connection, the objective of our present study was to evaluate the effectiveness of using pituitary extracts from goat (*Capra aegagrushircus*) and pig (*Sus scrofa domesticus*) as potential sources for the induced spawning of African catfish *C. gariepinus*.

2 | METHODOLOGY

2.1 Broodstock selection and conditioning

A total of 15 female and 15 male *Clarias gariepinus* breeders (647.33 ± 59.22 g) were obtained from the Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Selection of broodstock was administered following the desirable characteristics such as: male breeders with a vascularised and protruding genital papilla; female breeders with plump, soft and elastic abdomen. Male and female breeders were stocked separately into 500 L conditioning tanks to avoid unnecessary breeding. Each tank was supplied with flow-through water system and artificial aeration.

2.2 Pituitary extract preparation and injection

Three different sources of pituitary extracts were examined i.e. African catfish, goat and pig. Pituitary extract form African catfish at 4 mg kg^{-1} body weight served as the control treatment (Mosha and Mlingi 2018). Additionally, the dosages of both 4 mg kg^{-1} and 200 mg kg^{-1} body weight from goat and pig pituitary extracts were used as the other treatments. Average weight of a single pituitary gland from goat and pig were 198.34 ± 11.21 g and 215.01 ± 9.92 g respectively, which served as the basis for the use of the dosage 200 mg kg^{-1} body weight. Collection of pituitary glands of goat, pig and African catfish was assisted by the City Veterinary Office of Science City of Muñoz, Nueva Ecija, Philippines to assure that the collected samples were authentic and proper protocols on the collection process were strictly followed in line with the animal welfare experimentation. Pituitary glands were obtained from mature donor animals (1-year old for African catfish; 8-month to 12-month old for goat; and 6-month old for pig). By the method of acetone preservation, the extracted pituitary glands were preserved in a vial with fresh acetone for 36 – 48 h. During this period, the acetone was changed 2 – 3 times every 8 – 12 h interval for proper de-fatting and dehydration (Sharad *et al.* 2015). After preservation, the pituitary glands were macerated in a mortar and pestle by adding 1 mL of 0.9% saline solution. The homogenised mixture was drawn into a hypodermic syringe for injection. Each female breeder was single injected following the designated dosage per treatment with three replications. Injection was administered intramuscularly above the lateral line and below the anterior part of the dorsal fin. Each female was returned to the 100 L conditioning tanks after the injection of pituitary extracts. The actual experimentation was conducted at Freshwater Aquaculture Center, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines in June 2019.

2.3 Stripping and fertilisation

Dry method fertilisation was used in the study. Fertilisation was done using a sex ratio of 1 : 1 (M : F). Stripping of the females was carried out by wiping the genitalia and by gently pressing the abdomen from the pectoral fin towards the genital papilla. The stripped eggs were directed into a clean and dry plastic container. From each female breeder, a sample of 5 g was set aside in a petri plate and weighed for the determination of fecundity. Collection of milt extract was done through dissection by cutting the vent part of the anaesthetised male breeder to obtain the testes. The testes were cut into small pieces allowing the milt to ooze out. After collection, sperm activation was done by adding 5 mL of distilled water and sperm motility was verified under compound microscope (100X). The milt was mixed to the stripped eggs using clean feather for 1 min and rinsed by adding distilled water. The fertilised eggs were incubated at 26 – 28°C temperature in a 5 L circular basin with a flow-through water system. A framed fine mesh net was placed inside the basin to suspend the eggs within the water column and as a way to avoid settling of eggs at the bottom.

2.4 Determination of fecundity, relative fecundity, fertilization rate and hatching Rate

Latency period was recorded from the time female fish was injected until the eggs were ready to be stripped. The stripped eggs from each female fish were weighed. Eggs (5 g) from each female were collected and set aside in a petri plate for the determination of fecundity, relative fecundity, fertilisation rate and hatching rate. Fecundity (total number of eggs) and relative fecundity (number of eggs g^{-1} of female body weight) were determined using the following formulas:

$$\text{Fecundity} = \text{Total weight of eggs} \times \text{number of eggs } g^{-1}$$

$$\text{Relative Fecundity} = \frac{\text{Total number of eggs}}{\text{Body weight of brooder}} \times 100$$

Fertilisation rate was determined by counting the fertilised and unfertilised eggs under dissecting microscope. Sampled eggs were observed after 24 h of incubation. Unfertilised eggs are characterised by having an opaque appearance while the fertilised eggs are transparent with notable embryo. The fertilisation rate was computed as follows:

$$\text{Fertilisation Rate} = \frac{\text{Total number of fertilised eggs}}{\text{Total number of fertilised and unfertilised eggs}} \times 100$$

Monitoring of the hatching time started when the spermatozoan solution was distributed to the eggs until the time the eggs were hatched (100% hatching rate). A sample of eggs (5 g) for the determination of hatching rate was placed in a 5 L circular basin with continuous water inflow. Hatched larvae were counted to determine the total number of hatched eggs following this formula:

$$\text{Hatching Rate} = \frac{\text{Total number of hatched larvae}}{\text{Total number of sampled eggs}} \times 100$$

2.5 Data analysis

All percentage data were transformed using arcsine transformation and analysed using one-way analysis of variance (ANOVA). Comparison of mean values was carried out using Duncan's Multiple Range tests at the significant level ($p < 0.05$). SPSS Statistics 17.0 was used for the statistical analysis.

3 | RESULTS

The study revealed that all 15 African catfish female breeders administered with pituitary extracts from African catfish (control), goat and pig were induced to spawn and attained ovulation within 10 – 12 h post injection. Results in terms of relative fecundity were not significantly different among treatment groups showing that any of the pituitary extracts used will likewise yield a comparable result (Table 1). Though result did not significantly affect the dosage used, higher values were generated from 4 mg kg^{-1} body weight of both goat and pig pituitary extracts. This may imply the recommended dosage using these two sources. Similar pattern of result was generated in terms of fecundity with a total number of eggs spawned ranged from 36200 – 67600.

Recorded fertilisation rate ranged from 54.12% to 69.3%. Fish supplied with goat pituitary extract at 4 mg kg^{-1} body weight dosage was not significantly different to fish administered with African catfish pituitary extract with the same dosage (control). Increasing the dosage using goat pituitary extract may lead to percentage reduction of fertilised eggs as significantly lower ($p < 0.05$) fertilisation rate was attained by higher dosage (200 mg kg^{-1} body weight) compared to lower dosage (4 mg kg^{-1} body weight). Further, similar result was obtained using pig pituitary extracts irrespective of the dosage used (Table 1).

In terms of the hatching period, hatching time was recorded 24 – 36 h after fertilisation. Yolk absorption among hatched eggs was observed until fourth day of incubation. Hatching rate ranged from 33.40% to 34.35% in which pituitary extracts from goat significantly attained the highest value ($p < 0.05$) (Table 1).

4 | DISCUSSION

Gonadotropin releasing hormone (GnRH) which is the best available biotechnological tool for the induced breeding of fish is the key regulator and central initiator of reproductive cascade in all vertebrates (Bhattacharya *et al.* 2002). There is a previous report on the isolation of hypothalamic extracts obtained from porcine or pig which

is considered polypeptide that has both luteinising hormone-releasing hormone (LH-RH) and follicle stimulating hormone-releasing hormone (FSH-RH) activity (Schally *et al.* 1971). Similarly, the events of the estrous cycle in goat are controlled by the relationships of the hypothalamic releasing hormones, gonadotropins, and ovarian hormones. The GnRH comes from the hypothalamus of the brain and causes the pituitary gland to release FSH and LH (Senger 1997). It is assumed that the potential of using goat and pig pituitary extracts on the spawning induction of African catfish is due to the characteristics of pituitary glands to produce LH and FSH which are gonadotrophic hormones that trigger ovulation and increase oestradiol production in fish (Holesh and Lord 2019). There were previous studies reported that animal-source pituitary extracts can successfully induced the spawning of various fish species. The study of Oka (2005) reported that common carp (*Cyprinus carpio*) was effectively induced to spawn using cattle's pituitary-gland. In addition, chicken pituitary extracts have been applied and successfully stimulated the ovulation of *C. gariepinus* (Taufek *et al.* 2009), common carp (*C. carpio*) (Kruger *et al.* 1984), goldfish (*Carassius auratus*) (Andalusia *et al.* 2008) and seurukan fish (*Osteochilus vittatus*) (Muchlisin *et al.* 2014).

TABLE 1 Weight of female brooder, fecundity, relative fecundity, fertilisation rate and hatching rate of induced spawned *Clarias gariepinus* using pituitary extracts from African catfish (*Clarias gariepinus*), goat (*Capra aegagrushircus*) and pig (*Sus scrofa domesticus*).

Parameters	Pituitary extracts sources and dosages				
	CPG-4	**GPG-4**	**GPG-200**	**PGP-4**	**PGP-200**
Weight of female brooder (g)	640±17.3[a]	653.3±83.9[a]	633.3±97.1[a]	640±36.1[a]	670±72.1[a]
Fecundity	39500±22331.9[a]	49600±41155.7[a]	36200±26082.7[a]	67600±11240[a]	43000±6594.7[a]
Relative fecundity	62±34.2[a]	71±50[a]	62±50.1[a]	105±13.5[a]	65± 16.1[a]
Fertilisation Rate (%)	69.0±0.8[c]	66.4±0.9[c]	54.1±2.1[a]	58.9±0.4[b]	58.6±0.8[b]
Hatching Rate (%)	34.6±0.00[b]	34.7±0.9[c]	34.8±2.1[c]	33.5±0.4[a]	33.4±0.8[a]

Note: Values (means ± SD) in the same row followed by different superscripts are significantly different ($p < 0.05$). CPG-4, African catfish pituitary extract using 4 mg kg^{-1} body weight dosage; GPG-4, goat pituitary extract using 4 mg kg^{-1} body weight dosage; GPG-200, goat pituitary extract using 200 mg kg^{-1} body weight dosage; PPG-4, pig pituitary extract using 4 mg kg^{-1} body weight dosage; PPG-200, pig pituitary extract using 200 mg kg^{-1} body weight dosage; n = 15, three experimental fish per treatment.

Both dosages used from the present study provided similar result in terms of egg quantity; however, lower dosage demonstrates advantage in terms of the number of breeders it can cater since utilisation of 4 mg kg^{-1} body weight is already adequate to induce the spawning of African catfish in contrast to higher dosage (200 mg kg^{-1} body weight). This result agreed with the findings of Mosha and Mlingi (2018) that exact dosage of 4 mg kg^{-1} body weight gave the highest eggs spawned for African catfish using homoplastic pituitary extract compared to those dosages that are lower or higher than 4 mg kg^{-1} body weight. Further, Michael *et al.* (2004) reported that high dosage application in inducing *Eleutherodactylus conqui* to spawn using different hormone mixtures resulted to onset occurrence of side effects to breeders such as hemorrhaging, ataxia and lethargy which compromised egg quality and quantity.

The fertilisation rate obtained serves as an indication on the effectiveness of pituitary extracts from goat and pig to induce the breeding process of African catfish. According to Rurangwa *et al.* (2004), fertilisation and hatching during induced spawning can be influenced by biological factors like the quality of milt which is a measure of the ability of sperm to successfully fertilise an egg and such ability mostly depends on qualitative parameters of milt composition of seminal fluid, milt volume, sperm density and sperm motility. The result obtained from the current study was similar to the study of Adebayo and Popoola (2008) wherein catfish pituitary extract gave a fertilisation rate of 73% higher than African bullfrog with 60.50% fertilisation rate. However, in the study of Gadissa and Devi (2013) the rate of fertilisation for female African catfish injected with carp pituitary extract was higher (80.53%) than with catfish pituitary homogenate (76.93%). Muchlisin *et al.* (2014) reported a fertilisation rate of 82.33% from chicken pituitary extracts which was lower compared to ovaprim, but better than oxytocin in induced spawning of *O. vittatuis*. The result may be attributed to species differences by having variations in habitat choices and culture systems on how they have been reared and the food they eat that might influence the activity of the endocrine system where the pituitary gland can be located.

Hatching time starts at the beginning where the spermatozoan solution was distributed to fertilise the eggs until the time the eggs were hatched. According to Potongkam and Miller (2006), hatching success of African catfish eggs occur after 36 to 48 h where from this time the yolk sac of larvae was totally absorbed. Hatching periods on the current study were observed within 24 – 36 h

of incubation, as this time duration, eggs were completely hatched. This result was similar to the study of Adebayo and Popoola (2008), wherein the hatching occurred 24 – 35 h after fertilisation.

Based on the present study, positive outcome in terms of the hatching rate of African catfish eggs was generated. Preliminary studies by Ataguba *et al.* (2012 and 2013) revealed that increasing broodstock size caused significant increase in hatching success. However, in the study of Maradun *et al.* (2018), they concluded that breeders with body weight ranging from 600 g successfully induced the spawning of African catfish and can provide high percentage of egg hatchability using ovulin hormone. In addition, better egg hatchability was recorded on the study of Aruho *et al.* (2016) using African catfish pituitary extract compared to commercially-available GnRH synthetic analogue with an anti-dopamine drug ([D-Arg6, Pro9-NEt])- sGnRH; 10 µg kg^{-1} + metoclopramide 20 mg kg^{-1}) in artificial spawning of *Barbus altianalis*. The results obtained from the recent study were higher than the findings of Olaniyi and Akinbola (2013) whereas, African catfish injected with African catfish pituitary extract attained a hatchability rate of 25.99%. This can be attributed due to the different broodstock size used without neglecting the efficacy of pituitary extract used for the induced spawning.

5 | CONCLUSIONS

Single intramuscular injection of goat (*Capra aegagrushircus*) and pig (*Sus scrofa domesticus*) pituitary extracts using the dosage 4 mg kg^{-1} body weight provided an acceptable outcome in terms of fecundity, fertility and hatchability of eggs which revealed to be as efficient as utilisation of pituitary extracts from African catfish. Thus, the current study suggests that goat and pig pituitary extracts can effectively use for the induced spawning of African catfish. To our knowledge, no previous study was reported in inducing African catfish to spawn using these two mammalian pituitary extracts hence, the results of this study provide preliminary information in terms of its spawning success.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

JSD: Conceptualisation, methodology, resources, data collection, data analysis, manuscript preparation, writing – review & editing, visualization, supervision; DSS: conceptualization, methodology, data collection, data analysis, manuscript preparation, writing – original draft; LJF: resources, writing – review & editing, visualization

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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