Adamawa State University Journal of Scientific Research
Volume 9 Issue 1, 2021; Article no. ADSUJSR 0901020
ISSN: 2705-1900 (Online); ISSN: 2251-0702 (Print)
http://www.adsujsr.com

Effects of Natural Pituitary and Synthetic (Ovaprim) Hormones on haematology of *Clarias gariepinus* injected at various hours of inducement in Mubi

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(Received in June 2021; Accepted in July 2021)

Abstract
This study was carried out to determine the effects of natural and synthetic hormones on *haematology of Clarias gariepinus* broodstock. 12 broodstock of mean weight of 1.7kg and mean length of 26cm were used for the experiment. One male and one female served as control, one male and one female for natural hormone and one male and one female for synthetic hormone. The brooders were injected at the same time at 27^{o}C water temperature. Blood samples were collected at 00hrs, 6hrs, 12hrs and 24hrs of injection from all the treatment and control. Blood samples were analysed at the end of the experiment. Statistical analysis was used to analyse the data collected on haematological parameters. At the end of the research it was discovered that there is significant variation in all the haematological parameters between the natural and synthetic hormone and between the male and female fish with the female fish having the highest value of almost all the haematological parameters during the course of the study. The use of natural hormone is therefore recommended in aquaculture.

Key words: Natural hormone, Ovaprim, haematological parameters, *Clarias gariepinus* and Mubi.

Introduction

Over the last few decades, hormonal administration techniques have been used to induce final oocyte maturation and spawning in fishes which allowed the reproduction in controlled conditions (Marimuthu *et al*., 2007 and 2009; Marimuthu and Haniffa, 2010). Moreover, induced breeding techniques have significantly contributed a lot to the expansion and diversification of the aquaculture industry (Zohar and Mylonas, 2001). The injection of different spawning agents in fish is adopted for successful ovulation and collection of eggs. Traditional methods of induced spawning in fish are based on the injection of GtH-II from different sources, including extract of carp pituitary gland, partially purified fish GtH-II and mammalian GtH, especially human chorionic gonadotropin (HCG) (Lam, 1982; Donaldson and Hunter, 1983; Peter *et al*., 1988; Zairin *et al*., 1992; Goswami and Sharma, 1997). Several researchers have shown that pituitary hypophysation is effective in inducing the spawning of *Clarias gariepinus*, also synthetic hormones have been used to induce maturation, spawning and ovulation by using GnRH Analog with Dopamine Antagonist and Luteinizing hormone releasing hormone (LHRH). Ovaprim, which is a combination of Salmon GnRH analog combined with a dopamine, has proved to be extremely successful in breeding of *C gariepinus*. Artificial propagation of fish is the most promising and reliable way of ensuring availability of good quality fish seed all year round and sustainability of the aquaculture industry. The GnRHa and domperidone are the most popular compounds for induction of ovulation and spermiation in various fish species. The introduction of GnRH analogues has been proven to be efficient in inducing maturation and spawning in many fish species (Tamaru *et al*., 1988; Zohar, 1988; Slater *et al*., 1995; Berlinsky *et al*., 1996). Increase in fish production can be achieved by understanding the physiology of the fish species. Since blood tissue reflects physical and chemical changes in the body, accurate information can be obtained on the general

metabolic and physiological status of fish when injected with chemical compound. Fish exposure to chemical compound can increase or reduce the haematological parameters. The use of this chemical on *C gariepinus* need to be accessed to know whether the chemical reduce or increase the haematological parameters of the fish.

Materials and Methods

Twelve broodstock of an average weight of 1.7kg and mean length of 26cm of *Clarias gariepinus* six males and six females were purchased from a private fish farm in Mubi, Adamawa State. The broodstock were transported to the Department of Fisheries and Aquaculture in a 50 litre jerry can. The fish were allowed to rest for the period of 3 days during which vital feed of 42% C.P was fed at 1% body weight. Six males and six females were injected with 0.25ml/kg body weight while female were injected with 0.5ml/kg body weight three of each with the synthetic (Ovaprim) and natural pituitary gland hormone. Latency period was oserved.

Collection of Blood Samples

Blood samples were collected from both male and female brooders from each of the natural and synthetic hormone before and after injection, at 6 hours, 12 hours and 24 hour of injection. 0.1- 0.2ml blood samples were collected through cardiac puncture using 2ml disposale heparinised syringe treated with EDTA. Blood analysis were determined according to the method described by Svoboda *et al.,* (1991).

Determination of Packed Cell Volume (PCV)

The Packed Cell Volume was measured after placing sealed micro-haematocrit tube in a centrifuge at 10,500 rpm using micro-haematocrit reader and expressed as percentage (Svoboda *et al.,* 1991).

Haemoglobin Estimation

Haemoglobinometer was used for haemoglobin estimation based on acid haematin method (SAHLI).

Haemoglobin = $\frac{value\ obtained}{100} \times 17.2\ mg/100ml$

Red Blood Cell (RBC), White Blood Cell (WBC) Count

Haemocytometer was used in both the red and white blood (RBC and total WBC) count. The blood diluting fluid was prepared as described by Svoboda *et al* (1991).

The blood cells was counted on the counting chamber of haemocytometer with the aid of compound microscope:

- RBC = No. of cells Counted × 3 × 10 × 200 ($10^6 mm^3$)
- WBC = No. of cells Counted × 0 × 25 × 10 × 20 ($10^4 mm^3$)

Mean Corpuscular Volume (MCV)

MCV was calculated from the haematocrit value (PCV % and the erythrocyte count ($Ermm^3$).

MCV (μ^3) = $\frac{PCV}{Er} \times 10$

Mean Corpuscular Haemoglobin (MCH)

MCH was calculated from the result of Haemoglobinometer value (Hb %) and the erythrocyte count and was expressed in picograms (Pg).

MCH (Pg) = $\frac{Hb}{Er} \times 10^2$

Mean Corpuscular Haemoglobin Concentration (MCHC)

This was obtained using the formula:

MCHC (%) = $\frac{Hb}{Er} \times 100$

Data collected were analyzed using one way Analysis of Variance (ANOVA) using SAS while mean were separated using Duncan Multiple Range Test. (Duncan, 2006)

Result and Discussion

The result of Hb (Heamaglobin estimate) in female injected with ovaprime (synthetic) hormone, recorded the highest value of 7.90 ± 0.10 at 6hours of injection. While PCV (packed cell volume) of 24.00 ± 0.00 was recorded at 12hours of injection. Natural pituitary recorded the least value of 19.00 ± 0.00 and 6.90 ± 0.00 respectively. Schalm, (1975) stated that the normal ranges of PCV of some domestic animals are between, 37-55. Nutritional deficiencies, stress, diseases, pollution, number of red cell in blood, plasma volume and body weight of fish usually affect the PCV and Hb range. Smith (1982) showed that there was significant decrease in blood cell parameters

including PCV of rainbow trout during infection with infectious haematopoietic necrosis.

Table 1: Haematological Indices of *C gariepinus* Induced With Natural Pituitary

Parameters	Sex	Hours of Injection 0 hours	6 hours	12 hours	24 hours
Hb (g/dc)	M	7.50 ± 0.21^{b}	7.00 ± 0.31^{a}	6.90 ± 0.21^{b}	6.90 ± 0.21^{b}
	F	6.70 ± 0.11^{c}	6.90 ± 0.170^{b}	7.10 ± 0.19^{a}	7.20 ± 0.13^{a}
PCV (%)	M	22.00 ± 0.60^{c}	21.00 ± 0.00^{a}	18.00 ± 0.00^{c}	20.00 ± 0.00^{b}
	F	20.00 ± 0.00^{c}	19.00 ± 0.00^{b}	20.00 ± 0.00^{c}	21.00 ± 0.00^{a}
WBC ($\times 10^{12}/L$)	M	2.40 ± 0.31^{b}	2.10 ± 0.27^{b}	2.20 ± 0.21^{a}	2.10 ± 0.23^{b}
	F	2.40 ± 0.23^{b}	2.40 ± 0.21^{b}	2.40 ± 0.18^{b}	2.50 ± 0.20^{a}
RBC ($\times 10^{12}/L$)	M	2.50 ± 0.17^{a}	2.00 ± 0.20^{b}	2.50 ± 0.20^{a}	2.00 ± 0.20^{b}
	F	2.50 ± 0.21^{a}	2.60 ± 0.22^{a}	2.50 ± 0.19^{a}	2.50 ± 0.23^{a}
MCV (FL)	M	78.50 ± 0.67^{c}	84.00 ± 0.71^{a}	75.00 ± 0.80^{b}	74.00 ± 0.27^{b}
	F	79.20 ± 0.48^{c}	77.00 ± 0.63^{a}	76.00 ± 0.19^{b}	75.00 ± 0.20^{c}
MCH (Pg)	M	26.00 ± 0.12^{c}	28.00 ± 0.08^{a}	28.00 ± 0.11^{a}	25.50 ± 0.27^{b}
	F	27.90 ± 0.21^{a}	26.50 ± 0.17^{b}	28.40 ± 0.18^{b}	25.70 ± 0.23^{c}
MCHC (%)	M	34.00 ± 0.17^{a}	33.30 ± 0.23	37.80 ± 0.17	34.50 ± 0.23
	F	35.20 ± 0.11^{a}	34.50 ± 0.19	37.30 ± 0.21	34.30 ± 0.00

Means in the same row having the same super script do not differed significantly (P>0.05)

Table 2 Heamatological Indices of *C gariepinus* Induced with Ovaprime Hormone

Parameters	Sex	Hours of Injection 0 hours	6 hours	12 hours	24 hours
Hb (g/dc)	M	7.50 ± 0.21^{b}	7.20 ± 0.31^{c}	7.80 ± 0.31^{a}	7.60 ± 0.20^{b}
	F	6.70 ± 0.11^{c}	7.90 ± 0.10^{b}	7.40 ± 0.32^{a}	6.90 ± 0.30^{c}
PCV (%)	M	22.00 ± 0.60^{c}	23.00 ± 0.00^{b}	24.00 ± 0.00^{a}	23.00 ± 0.00^{b}
	F	19.00 ± 0.00^{c}	21.00 ± 0.00^{b}	22.00 ± 0.00^{a}	21.00 ± 0.00^{b}
WBC ($\times 10^{12}/L$)	M	2.40 ± 0.31^{b}	2.30 ± 0.11^{c}	2.20 ± 0.13^{c}	2.50 ± 0.17^{a}
	F	2.30 ± 0.23^{b}	2.40 ± 0.28^{b}	2.70 ± 0.24^{a}	2.70 ± 0.21^{a}
RBC ($\times 10^{12}/L$)	M	2.50 ± 0.17^{b}	2.50 ± 0.31^{b}	2.70 ± 0.24^{a}	2.70 ± 0.24^{a}
	F	2.50 ± 0.21^{b}	2.50 ± 0.24^{b}	2.40 ± 0.18^{b}	2.50 ± 0.20^{b}
MCV (FL)	M	78.50 ± 0.67^{c}	42.0 ± 0.21^{b}	96.00 ± 0.36^{a}	95.80 ± 0.21^{a}
	F	79.20 ± 0.48^{c}	80.70 ± 0.21^{b}	81.50 ± 0.41^{b}	84.00 ± 0.11^{a}
MCH (Pg)	M	26.00 ± 0.12^{c}	28.80 ± 0.21^{b}	31.20 ± 0.13^{a}	31.70 ± 0.23^{a}
	F	27.90 ± 0.21^{a}	27.00 ± 0.23^{a}	27.00 ± 0.17^{a}	27.60 ± 0.19^{a}
MCHC (%)	M	34.00 ± 0.17^{a}	31.30 ± 0.31^{c}	32.50 ± 0.14^{c}	33.00 ± 0.32^{b}
	F	35.20 ± 0.11^{a}	33.30 ± 0.21^{b}	33.60 ± 0.19^{b}	32.80 ± 0.18^{c}

Means in the same row having the same super script do not differ significantly ($P > 0.05$)

Male fish injected with synthetic (Ovaprim) hormone after 12 hours of injection, recorded the highest mean WBC (white blood cell count) of 2.70 ± 0.24, and the least value of 2.10 ± 0.00 was recorded in male fish injected with natural hormone at 6 and 12 hours of injection. It was observed that haematological indices have different sensitivity to various environmental factors and chemicals. White blood cell count of all the brooders showed variation in all the hours of injection. Thus, animals with low white blood cells are exposed to have high risk of disease infection, while

those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases and stress. (Soetan *et al*., 2013). Also high white blood cell count enhance adaptability to local environmental and disease prevalent conditions (Kabir, *et al.,* 2011; Okunlola, *et al.,* 2012; Iwuji and Herbert, 2012; Isaac *et al.,* 2013). Erythrocyte value of the present studies shows variation in both the natural and synthetic hormone, from 00 hours to 24 hours of injection with the highest value of 2.70± 0.24 at 12 hour and 24 hours of injection in female that was injected with synthetic hormone to the least value of 2.00± 0.20 at 6 and 12 hours of injection in male injected with natural hormone. The number of red blood cell per $10^{12}/L$ of blood varies among animal species, age, physical condition and activities. Firdaus *et al.* (1996) showed that haemotocritic values increase with dietary protein supplementation while the erythrocyte sedimentation rate (ESR) showed a marked decline in fresh water catfish. The count of red blood cells is quite a stable index and the fish body tries to maintain this count within the limits of certain physiological standards using various physiological mechanisms of compensation. Haematological studies on the effect of nutrition, infectious diseases and pollutants also stated same in study of Rehulka, (2002). The result also revealed that erythrocytes are the major and reliable indicators of various sources of stress which is in line with the findings of Rainza-paiva *et al.,* (2000); O'Neal and Werich, (2001). There is variation in all the haematological parameters between the natural and synthetic hormone and between the male and female fish with the female fish having the highest value of almost all the haematological parameters during the course of the study. It was be concluded that synthetic hormone injection during breeding of African cat fish has more effect on the haematological parameters of the fish than natural hormone. It is therefore recommended that the use of natural hormone in fish breeding should be encouraged and protracted for maximum ovulation and hatchability.

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