EFFECTS OF *Xylopia aethiopica* POD POWDER AS A FEED ADDITIVE ON HAEMATOLOGICAL AND REPRODUCTIVE PARAMETERS OF AFRICAN CATFISH (*Clarias gariepinus*) (Burchell, 1822)

B. Maliki Ibrahim[1,2,5], E. Miegoue[1], F. Djitie Kouatcho[2*], C. Bouba[2], I. Abdoullahi[5], S. Bagari Iya[6], A. Ali Moussa[5], B. Songmo[1], M. Burducea[3], R.M. Radu-Rusu[4]

[1]Department of Animal Sciences, Faculty of Agronomy and Agricultural Sciences. University of Dschang, Cameroon
[2]Department of Sciences and Techniques of Biological Agriculture, Faculty of Sciences, University of Ngaoundere, Cameroon
[3]Alexandru Ioan Cuza University of Iasi, Romania
[4]Faculty of Food and Animal Sciences, "Ion Ionescu de la Brad" Iasi University of Life Sciences, 8 Mihail Sadoveanu Alley, 700489 Iasi, Romania
[5]Institute of Agricultural Research for development (IRAD), Maroua Agricultural Research Center, Cameroon
[6]Institute of Agricultural Research for development (IRAD), Wakwa Agricultural Research Center, Cameroon

Abstract

In order to contribute to the improvement of the productive potential of African catfish through the use of plants with medicinal properties, a study was carried out from November 2023 to May 2024 at the Maroua Agricultural Research Center (CRA-Maroua), Diamaré Division, Far North Region (Cameroon). It focused on the effect of the inclusion of Xylopia aethiopica *pod powder as a feed additive on the reproductive and haematological parameters of* Clarias gariepinus. *To this end, a total 600 of a 4-week-old* C. gariepinus *fries with an average weight of 4g were randomly distributed in 151m^3 tanks each with 40 fingerlings. They were submited to 5 treatments consisting of concentrated feed with added different level of Guinea pepper pod powder (*X. aethiopica*): T0%; T0.25%; T0.5%; T0.75% and T1%* X. aethiopica*. The animals were fed 02 times a day (7H and 18H), at 5% of their biomass. Results showed that incorporation of* X. aethiopica *at 0.5% into the diet resulted in the highest egg weight (18.17±0.78g), number of oocytes/g of total eggs weight (7350.00±382.02) and absolute fecundity (133609.04±9449.34) at 24 weeks of rearing. In addition, the highest testicular value (3.54±0.34g) was observed at T0.25%. On the other hand,* X. aethiopica *pod powder had no significant effect on the relative fertility, oocyte diameter and gonado-somatic index of* C. gariepinus. *In addition, lymphocytes, white and red blood cells, haemoglobin and haematocrit increased significantly with the level of incorporation of* X. aethiopica. *On the other hand, MCV (Mean corpuscular volume) and MCH (Mean haemoglobin concentration) decreased significantly. It was thus concluded that* X. aethiopica *pod powder can be incorporated at 0.5% to improve the reproductive performance and blood quality of African catfish.*

Key words: *Xylopia aethiopica, feed additive, hematology, reproduction, African catfish Clarias gariepinus*

*Corresponding author: franckdjitie@gmail.com
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INTRODUCTION

Aquaculture is the fastest-growing form of animal production in the world [11][16]. In Cameroon, as in many other countries, fish is the main source of animal protein and essential trace elements, which are invaluable for nutritional balance and health. It accounts for around 40% of animal protein intake and 9.5% of the population's total requirements [6]. Among the fish species appreciated in Cameroon, *Clarias gariepinus* stands out for its high taste value, rapid growth rate, aerial respiration system that enables it to survive in oxygen-poor waters, resistance to handling and farming as a source of income for farmers [14][24]**.** Despite these advantages, fish constantly receive chemical substances and pathogenic bacteria in their digestive tract, which may come from the water, the feed or their faeces. These are likely to unbalance their intestinal flora, weaken the immune system and induce low productivity [5][21] [25]. Concerned about this problem, several researchers have proposed solutions, including feed additives in general and phytobiotics in particular. For example, [27] evaluated gametes quality, haematological and biochemical parameters of *Clarias gariepinus* brood stocks fed with varying concentrations of *Cissus populnea* (CP) and *Securidaca longepedunculata* (SL). Fish were fed with Diet 1 (control, 0 ml plant extract), Diet 2 (0.5 ml/kg CP), Diet 3 (1.0 ml/kg CP), Diet 4 (0.5 ml/kg SL), Diet 5 (1.0 ml/kg SL) and Diet 6 (0.5 ml/kg CP + 0.5 ml/kg SL) for 90 days. The results of haematocrit and red blood cell of treated groups were relatively similar to the control. The white blood cell and haemoglobin values were lower than the control group while the mean corpuscular volume, mean corpuscular haemoglobin were significantly difference across treatments however ; an elevated glucose level was recorded in the treated groups. Also, the mean platelet volume and platelet distribution width were relatively similar across treatments. The serum testosterone, progesterone and estrogen were found to be higher in fish fed diet 2 (0.5ml/kg CP). *C. populnea* at 0.5 ml/kg significantly ($P<0.05$) improved eggs weight, fecundity, gonadosomatic index, % fertilization and hatchability. Male brood stocks fed diet 2 (0.5 ml/kg CP) recorded the highest values for sperm motility (56.67 ± 0.33%), milt count and volume (820.33 ± 0.33×10 6 /ml and 1.80 ± 0.05 ml respectively) across all groups. Similarly, fish fed diet 2 (0.5ml/kg CP) had the highest values for eggs weight (283.7±102.4g), fecundity (168,286 ± 57157), gonadosomatic index (32.59 ± 2.72), fertilization (62 ± 20.4%) and hatchability (62.92 ± 19.75%). The dietary supplementation of 0.5 ml/kg *C. populnea* extract highly enhanced the reproductive profiles of male and female *C gariepinus* brood. [27] reported that incorporating the aqueous extract of *Cissus populnea* leaves at 0.5ml/kg of feed improved oocyte weight and fertility in *C. gariepinus*. Incorporating plants with medicinal properties into the diet of *C. gariepinus* would therefore be an excellent way of improving its productivity. A number of plants with medicinal properties are used in traditional pharmacopoeia, but very few are known to be used in animal feed. One of these plants is Guinea pepper (*Xylopia aethiopica*).

Commonly known as 'Guinea pepper' or 'African pepper', *Xylopia aethiopica* is a plant in the Annonaceae family, native to Africa and growing up to 20 metres tall. This plant produces pods with antimicrobial, antioxidant and anti-inflammatory properties, which are an excellent spice in cooking and used in traditional pharmacopoeia for the treatment of a number of illnesses, including respiratory, bacterial and parasitic diseases [18][26]. Phytochemical analysis of *Xylopia aethiopica* pod powder revealed the

presence of bioactive compounds such as flavonoids, alkaloids, terpenoids and tannins, and mainly total phenols, which could improve the productivity of *C. gariepinus*. [8] reported that these bioactive compounds show several properties other than antimicrobial, antioxidant and anti-inflammatory properties that can improve intestinal health and strengthen the immune system of animals. The work of [18] reported that the use of 2g of *Xylopia aethiopica* pod powder in 200g of cereals (i.e. 1% of *X. aethiopica* pod powder per 100kg of cereals) improved the survival rate and egg hatchability of Niebe's sparrows (*Callosobruchus maculatus*). In addition, the work of [17] showed that *X. aethiopica* pod extract significantly increased haemoglobin concentration, cell volume and red blood cells compared with control in albino Wister rats.

The general aim of the present study was therefore to help improve the productive potential of catfish by incorporating plants with medicinal properties into their diet.

Specifically, the aim was to assess the effect of powdered *Xylopia aethiopica* pods on *Clarias gariepinus*:

- Reproductive parameters ;
- Haematological parameters.

MATERIAL AND METHOD

Presentation of the study area

The study carry out from November 2023 to May 2024 at the Maroua Agricultural Research Center (CRA-Maroua), at a northern latitude of 10°58‘and an eastern longitude of 14°29’. The Centre is located in the Diamaré department in the Far North region of Cameroon. It has an average altitude of 423 metres, average annual rainfall of 836 millimetres, and average minimum and maximum temperatures of 37°C and 42°C respectively. The soil is predominantly fine sand with a pH of 5.2 to 4.5.

Materials used

Animal material and habitat

The study involved 600 *Clarias gariepinus* fries aged of 4 weeks olds and weighing ranged from 4g to 6g. These were randomly divided into 15 identical batches of 40 similar subjects following a completely randomised design of 5 treatments repeated 3 times. The fish were reared in 15 plastic tanks of the following dimensions: 1 m long, 1 m wide and 1 m deep (Photo 1).

Photo 1: *C. gariepinus* fries in rearing tanks

Plant material

The plant material consisted of *Xylopia aethiopica* pods from eastern Cameroon (Photo 2a). The pods were transported to IRAD in Maroua, where they were dried in an oven at 40°C until they reached a constant weight and ground. Once ground, they were dried again, then sieved using a 0.9mm mesh diameter sieve. The powder (Photo 2b) obtained was analysed in the chemistry laboratory of the University of Dschang to determine its phytochemical composition.

a b

Photo 2: (a): Pods and (b): *X. aethiopica* powder

Experimental diets

Five experimental diets (R0, R1, R2, R3 and R4) were produced using different levels of incorporation (0%, 0.25%, 0.5%, 0.75% and 1%) of *Xylopia aethiopica* pod powder in the compound feed. These diets were extruded into 2 and 4 mm diameter granules. The composition and bromatological characteristics of the compound feed are summarised in Table 1.

Table 1 : Composition and bromatological characteristics of compound feed

Ingrédients (kg)	Proportion of ingredients
Maize meal	10
Soya cake	20
Groundnut cake	16
Fish meal 60	53
Peanut oil (litre)	1
Total (Kg)	**100**
Chemical composition as a percentage of dry matter	
Crude protein (%MS)	46,75
Metabolisable energy (Kcal/Kg)	2991,4
crude cellulose (%MS)	3,4
Fat (%)	10,56
Ash (%)	8,76
Price (Fcfa/kg)	**452**

Trial design

To carry out this study, 600 fries of *C. gariepinus* aged 4 weeks and with an average weight of 4g were randomly distributed in 15 tanks (1m x 1m x 1m) of 40 comparable individuals. The animals in each tank were reared in 300 l of water, changed every four days. They were submited to 5 treatments consisting of concentrated feed with added powdered Guinea pepper pods (*X. aethiopica*) : T0% ; T0.25% ; T0.5% ; T0.75% and T1% *X. aethiopica*. These animals were fed 02 times a day (7H and 18H), at 5% of their biomass [15]**.** To monitor water quality, the pH, temperature, salinity, TDS (Total Dissolved Solid) and EC (Electrical Conductivity) of the water were taken daily before each feed using an electronic multi-parameter ; dissolved oxygen, ammonia, nitrite and nitrate concentrations were determined once a week using analysis kits. In addition, the tanks were cleaned at the start of the trial and then every 02 weeks. After 24 weeks of rearing, the animals were fasted for 24 hours, then 10 subjects at random per treatment were taken to the animal production laboratory in Maroua. The body of each fish was first disinfected using cotton wool containing ethanol. The blood was then collected from the caudal fins using a syringe (1ml), placed in tubes with anticoagulant and analysed using a 'Sysmex' automatic analyser. Then, 10 fish (5 males and 5 females) of comparable weight per treatment were sacrificed and dissected. The testicles and eggs from each fish were removed in their entirety and weighed using a branded electronic balance with a precision of 0.1g. Next, the number of oocytes was determined by the mechanical dissociation method, which involves dissociating 1g of oocyte using hypertonic saline (0.9% Nacl) and gentle mechanical shaking, and were then counted. Oocyte diameter was assessed by averaging the distance over 10 oocytes mesured on millimetre paper [28]. The weight of each oocyte was obtained by counting the number of oocytes contained in 1g of layer weighed on a precision balance to the nearest mg [4]. Absolute fecundity, relative fecundity and the gonado-somatic index were also assessed using the guidelines of [9][28].

Data collected and parameters studied

- **Haematological parameters**

The following haematological parameters were determined using a 'Sysmex' automatic analyser: red and white blood cells, haemoglobin, haematocrit, lymphocytes, mean corpuscular volume,

mean haemoglobin concentration and mean corpuscular haemoglobin concentration.

➢ **Reproductive parameters**

The following parameters were evaluated:

- ***Number of oocytes***: The number of oocytes was determined by the mechanical dissociation method on 1g of oocyte, which consists of dissociating the oocytes from the eggs using a saline solution (0.9% Nacl) and gentle mechanical shaking. The oocytes were then counted [12].
- ***Oocyte diameter (mm)***: average distance over 10 oocytes measured on graph paper [28] ;
- ***Oocyte weight***: the weight of each oocyte was obtained by counting the number of oocytes contained in 1g of eggs weighed on a precision balance to the nearest mg [4] ;
- ***Absolute fertility***: is obtained by multiplying the total number of oocytes in 1g by the total eggs weight [9][28];
- ***Relative fertility*** : is obtained by dividing the total number of oocytes for an individual by its body weight [28] ;
- ***Gonado-somatic index (%)*:** (Weight of gonads (g) / weight of fish (g)) *100

Statistical analysis

The data were processed using Excel spreadsheets and analysed using SPSS version 23.0 computer software and subjected to one-factor analysis of variance (ANOVA) (treatment level). Means were separated using Duncan's test at the 5% threshold when their differences were significant [23].

The statistical model used was as follows:

$$Xij = \mu + \alpha i + eij;$$

Xij: observation on animal j having received ration or treatment i;
μ: Overall mean ;
αi: Effect of level of treatment i ;
eij: Residual error due to animal j having received ration or treatment i.

RESULTS

➢ **Effects on reproductive parameters of *Clarias gariepinus***

Table 2 presents the effect of incorporating Guinea pepper pod powder (GPPP) (*X. aethiopica*) into the diet on the reproductive parameters of *C. gariepinus*. From table 2 it come out that the inclusion of GPPP in the diet induced a significant increase ($P<0.05$) in testes weight except for the T1% treatments. The highest value (3.54±0.34) was observed with T0.25%, comparable ($P>0.05$) with T0.5% and T0.75%; the lowest (2.86±0.37) with T0%. Regarding total eggs weight, it was found that, the inclusion of GPPP induced a significant increase ($P<0.05$) in total eggs weight except treatments T0.75% and T1% which were comparable ($P>0.05$) at T0%. The highest value (18.17±0.78) was observed with T0.5%, comparable ($P>0.05$) to T0.25% and the lowest (14.46±0.72) with T1%, which in turn was comparable ($P>0.05$) to T0% and T0.75%. Regarding number of oocytes/g of total eggs weight, it was found that the addition of GPPP to the *Clarias gariepinus* diet induced a significant increase ($P<0.05$) in the number of oocytes/g of total eggs weight at T0.5% and the other treatments were comparable ($P>0.05$) at T0%. The highest value (7350.00±382) was obtained with T0.5% and the lowest (6607.33±280.52) with T1%, which remained comparable ($P>0.05$) at T0%; T0.25% and T0.75%. In addition, GPPP in the feed induced a significant ($P<0.05$) decrease in Oocyte Weight with T0.5%, but the other treatments were comparable ($P>0.05$) at T0%. The highest value (0.152±0.0065) was observed with T1%, comparable ($P>0.05$) at T0%; T0.25% and T0.75%; and the lowest (0.136±0.0072) with T0.5%. In addition, GPPP induced a significant increase ($P<0.05$) in Absolute Fertility except for the T0.75% and T1% treatments which were comparable ($P>0.05$) at T0%. The highest value (133609.04±9449.34) was recorded

with T0.5%, comparable (P>0.05) to T0.25%; and the lowest (95694.36±8272.28) with T1% which was comparable (P>0.05) to T0% and T0.75%. Furthermore, the incorporation of GPPP in the feed induced no significant effect (P>0.05) on Oocyte Diameter, relative fecundity, gonado-somatic index of *C. gariepinus* compared to T0%.

- **Effects on haematological parameters of *Clarias gariepinus***

Table 3 below shows the effect of incorporating Guinea pepper pod powder (GPPP) (*X. aethiopica*) into the diet on the haematological parameters of *C. gariepinus*. The addition of GPPP resulted in a significant increase (P<0.05) in the white blood cells (WBC) level, with the exception of T0.25% and T0.5%, which remained comparable (P>0.05) at T0%. The highest value (14.34±0.38) was observed with T1% which was comparable (P>0.05) to T0.75%, and the lowest (13.21±0.42) with T0.5%, comparable (P>0.05) to T0% and T0.25%. GPPP incorporation also resulted in a significant (P<0.05) increase in lymphocyte count, with the exception of the T0.5% treatment which remained comparable (P>0.05) to the control (T0%). The highest value (73.01±4.17) was obtained with T1% which was comparable (P>0.05) to T0.75%, and the lowest (62.37±4.43) with T0.5%, comparable (P>0.05) to T0%. In addition to this, the addition of GPPP also resulted in a significant (P<0.05) increase in red blood cells (RBC) with the exception of T0.5% which was comparable to the control treatment (T0%). The highest value (4.07±0.41) was observed with T1%, comparable (P>0.05) to T0.75%, and the lowest (3.07±0.58), recorded with T0%, comparable (P>0.05) to T0.5%. From the above, the addition of GPPP induced a significant (P<0.05) increase in haemoglobin (HGB) rate with the exception of T0.25% and T0.5% which remained comparable to T0%. The highest value (14.82±0.45) was obtained with T1%, comparable (P>0.05) to T0.75%; and the lowest (13.74±0.63) with T0%, comparable to T0.25% and T0.5%. Furthermore, the addition of GPPP resulted in a significant increase (P<0.05) in haematocrit (HCT) in all treatments compared to T0%. The highest value (25.01±1.16) was recorded with T0.75%, comparable (P>0.05) with T0.25%; T0.5% and T1%; and the lowest (20.83±2.04) with T0%. The same trend was observed for mean corpuscular haemoglobin concentration (MCHC). The highest value (60.44±5.76) was observed at T0.5%, comparable (P>0.05) at T0.25%; T0.75% and T1% and the lowest (35.66±24.56) at T0%. Furthermore, the addition of GPPP resulted in a significant decrease (P<0.05) in mean haemoglobin concentration (MCH) in all treatments compared to the control (T0%). The largest value (52.96±12.35) was observed with T0% and the smallest (36.71±3.66) with T1% which remained comparable (P>0.05) at T0.25%, T0.5% and T0.75%. Furthermore In contrast to the CVD rate, the addition of GPPP did not result in a significant (P>0.05) increase on CVD compared to the control.

DISCUSSIONS

- **Effects on reproductive parameters of *Clarias gariepinus***

Effects on testicular weight

Inclusion of PGPG induced a significant increase in testicular weight except for T1% treatments. The highest value was observed with T0.25%, the lowest with T0%. This could be explained by the fact that the total phenols in the PGPG and at very low doses in the feed would have reduced oxidative stress and improved testicular maturation by stimulation of testosterone, which would have led to hypertrophy of the testicular cells resulting in an increase in testicular weight [22].

Table 2: Reproductive parameters of *C. gariepinus* according to the different treatments

Reproductive parameters	Traitements					*P*
	T0%	T0.25%	T0.5%	T0.75%	T1%	
TW(g)	2.86±0.37c	3.54±0.34a	3.46±0.05ab	3.10±0.09abc	3.02±0.13bc	*0.026*
TEW(g)	15.35±0.45bc	17.52±0.59a	18.17±0.78a	15.76±0.70b	14.46±0.72c	*0.000*
NO/g of TEW	6811.00±158.37b	6791.67±263.61b	7350.00±382.02a	6705.00±177.30b	6607.33±280.52b	*0.046*
OD (mm)	1.23±0.05a	1.40±0.10a	1.36±0.15a	1.26±0.05a	1.23±0.05a	*0.153*
OW (mg)	0.147±0.0034a	0.147±0.0057a	0.136±0.0072b	0.149±0.0039a	0.152±0.0065a	*0.055*
AF	104642.23±5510.94c	119092.94±7579.28b	133609.04±9449.34a	105646.86±3852.69c	95694.36±8272.28c	*0.001*
RF	50.77±4.54a	43.16±1.61a	47.80±7.49a	48.27±3.51a	51.31±4.41a	*0.299*
IGS.F	0.114±0.0098a	0.111±0.0018a	0.117±0.0120a	0.113±0.0020a	0.112±0.0073a	*0.873*
IGS.M	0.025±0.0023a	0.025±0.003a	0.025±0.0013a	0.024±0.0009a	0.028±0.001a	*0.447*

a,b,c : Means bearing the same letters on the same line are not significantly different at the 5% threshold ; T0%: Concentrated feed without GPPP; T0.25% : Concentrated feed containing 0.25% GPPP; T0.5% : Concentrated feed containing 0.5% GPPP ; T0.75% : Concentrated feed containing 0.75% GPPP; T1% : Concentrated feed containing 1% GPPP; TW: testicular weight ; TWC : Total eggs weight ; NO/g of TEW : number of oocytes/g of total eggs weight; OD: oocyte diameter; OW: oocyte weight; AF: absolute fertility; RF: relative fertility; IGS. F: gonado-somatic index for females; IGS.F: gonado-somatic index for males.

Table 3: Haematological parameters of *C. gariepinus* recorded at 24 weeks of rearing according to the different treatments

Haematological parameters	Traitments					*P*
	T0%	T0,25%	T0,5%	T0,75%	T1%	
WBC(x10^9/µL)	13.45±0.37b	13.65±0.44b	13.21±0.42b	14.10±0.07a	14.34±0.38a	*0.000*
LYM(%)	62.83±3.91c	66.32±5.15bc	62.37±4.43c	70.33±4.22ab	73.01±4.17a	*0.001*
RBC(x10^6/µL)	3.07±0.58b	3.50±0.53ab	3.25±0.37b	3.90±0.48a	4.07±0.41a	*0.007*
HGB (g/dL)	13.74±0.63b	14.14±0.40b	13.88±0.44b	14.79±0.53a	14.82±0.45a	*0.001*
HCT(%)	20.83±2.04b	24.13±0.87a	23.16±2.58a	25.01±1.16a	24.76±1.44a	*0.002*
MCV (fL)	70.46±17.23a	70.41±12.15a	72.25±13.59a	64.76±6.42a	61.29±6.68a	*0.482*
MCH (pg)	52.96±12.35a	41.09±5.64b	43.07±4.82b	38.38±4.53b	36.71±3.66b	*0.004*
MCHC (g/dL)	35.66±24.56b	58.67±2.46a	60.44±5.76a	59.19±1.68a	60.10±4.97a	*0.004*

a,b,c: Means with the same letters on the same line are not significantly different at the are not significantly different at the 5% threshold; T0%: Concentrated feed without GPPP; T0.25%: Concentrated feed containing Concentrated feed containing 0.25% GPPP; T0.5%: Concentrated feed containing containing 0.5% GPPP; T0.75%: Concentrated feed containing 0.75% GPPP; T1%: Concentrated feed containing 1% GPPP; WBC: white blood cells; RBC: red blood cells; HGB : haemoglobin; HCT: haematocrit; LYM: lymphocytes; MCV: mean corpuscular volume; MCH: mean haemoglobin concentration; MCHC: mean corpuscular haemoglobin concentration.

Effect on eggs weights and absolute fecundity

Incorporation of PGPG into the diet of *Clarias gariepinus* induced a significant increase in egg weight and absolute fecundity. The highest values of egg weight and absolute Fertility were observed with T0.5%. Low-dose PGPG would have improved the reproductive health of the fish by reducing oxidative stress in the eggs and stimulating oestradiol production, which would have promoted better growth and ovular development on the one hand, and the relative fecundity rate on the other. This is in agreement with the work of [19] who showed that the incorporation of *Carpolobia lutea* leaves at 0.5% significantly increased egg weight and absolute fecundity.

Effect on the number of oocytes/g of total eggs weight

Incorporation of GPPP into the diet of *C. gariepinus* induced a significant increase in the Number of oocytes/g of total eggs weight. The highest number of oocytes/g of total eggs weight was obtained at T0.5%. At low doses, GPPP would have improved reproductive health and stimulated the ovaries to produce significantly more oocytes. These results are similar to those of [19] who reported the highest value obtained with 0.5% *Carpolobia lutea* leaves.

Effect on oocyte weight

The incorporation of GPPP into *Clarias gariepinus* feed only produced a significant decrease in oocyte weight at T0.5%. GPPP incorporated at a medium rate would induce a decrease in Oocyte Weight. This decrease would be due to competition for nutritive resources linked to the number of oocytes. In fact, when the number of oocytes increases, their weight decreases due to the limited availability of resources during the ovarian development. This unavailability of resources is thought to have influenced oestrogen levels, thus disrupting oocyte growth and leading to a reduction in oocyte weight [7]. This is in agreement with the work of [19] who revealed that the incorporation of Datura stramonium seeds at gradual levels (0.5% and 1%) in the diet of *C. gariepinus* would significantly reduce the weight of the oocytes.

- **Effect on haematological parameters of *Clarias gariepinus***

Effect on white blood cells

The incorporation of guinea pepper pod powder (GPPP) induced a significant increase in white blood cells (WBC) levels in juvenile *Clarias gariepinus*. This could be explained by the fact that GPPP is rich in total phenols, which would have stimulated the production of white blood cells, thereby boosting the immune response. The highest rate of WBC was obtained with 1% GPPP. These results are close to those obtained by [1] who reported the value of WBC, with 1% *Aloe barbadensis* leaf. Similarly, the work of [10] reported the highest WBC in *C. gariepinus* fed with 1% garlic (*Allium sativum*).

Effect on lymphocytes

Incorporation of GPPP induced a significant increase in lymphocyte count. The highest value was obtained with T1%. This is thought to be due to the concentration of the bioactive compound contained in the phyto-additive, which induced lymphocyte proliferation, there by improving the immune response. These results are comparable to those of [1] who reported 62.67±0.88% lymphocyte with 1% *Aloe barbadensis* leaf. Similarly, [10] obtained the highest lymphocyte concentration with 1% garlic.

Effect on haemoglobin

In addition, incorporation of GPPP also induced a significant increase in haemoglobin. The highest value was obtained with T1%. The high concentration of total phenols in the GPPP and at a high

level in the feed would have induced oxidative stress, which would have stimulated the fish to produce many more red blood cells to compensate for the decrease in oxygen due to oxidative damage. These results are similar to those of [3] who reported 15.1±0.14g/dL haemoglobin with 1% *Moringa oleifera* leaf extract. This could be due to the fact that the alkaloids, terpenoids and flavonoids contained in GPPP stimulate the bone marrow to synthesise red blood cells efficiently.

Effect on red blood cells

Incorporation of GPPP induced a significant increase in red blood cell count. The highest value was obtained with T1%. GPPP incorporated at a high level would have induced an increase in tissue oxygenation in fish by increasing the number of red blood cells. The alkaloids, flavonoids and total phenols contained in GPPP had an erythropoiesis-inducing effect. These results are in agreement with the work of [1] who reported 2.73 ± 0.06 $\times 10^6$ L-1 of red blood cells with 1% *Aloe barbadensis* leaf. A similar trend was observed by [2] who obtained the highest rate 1.19 ± 0.01 with 1% *African Aspilia* leaf powder

Effect on haematocrit (HCT)

From the above, incorporation of GPPP induced a significant increase in HCT of *Clarias gariepinus*. The highest value was obtained with T0.75%. The incorporation of GPPP at a high rate would have stimulated the bone marrow to produce many more red blood cells, resulting in an increase in haematocrit. These results are similar to those of [20], who showed that incorporating 0.5% *Ageratum conyzoides* leaves into feed increased haematocrit values. On the other hand, [10] recorded the highest value with 1% honey. These observed differences could be due to the different concentration of bioactive compounds in the phyto-additives used.

Effect on mean haemoglobin concentration (MCH) and mean corpuscular haemoglobin concentration (MCHC)

On the other hand, incorporation of GPPP into the diet of *Clarias gariepinus* induced a significant decrease in MCH levels. The highest value was obtained with T0% and the lowest with T1% of GPPP. This decrease could be justified by the fact that, at a high dose in the feed, the total phenols contained in GPPP would have inhibited the growth of red blood cells, resulting in a decrease in haemoglobin. This result is in agreement with [13] who reported that incorporation of *Ageratum conyzoides* leavesinto the feed reduced haemoglobin in the blood. Unlike MCH, GPPP caused a significant increase in MCHC. This could suggest a high concentration of haemoglobins in the red blood cells. The highest value was obtained with T0.5%. This is in agreement with the work of [2] who reported that the incorporation of 0.5% *African Aspilia* leaf powder increased the MCHC level of *C. gariepinus.*

CONCLUSIONS

At the end of this study which focused on the effects of the level of incorporation of the powder of the pods of Guinea pepper (*Xylopia aethiopica*) on the parameters of reproduction and some haematological parameters of the African catfish (*Clarias gariepinus*) (Burchell, 1822), it emerges that: the incorporation of the powder of *X. aethiopica* powder at 0.5% significantly increased the weight of eggs and testes, the number of oocytes/g of total eggs weight and absolute fecundity while decreasing the weight of *C. gariepinus* oocytes. In contrast, *X. aethiopica* pod powder had no significant effect on the relative fertility, oocyte

diameter and gonado-somatic index of *C. gariepinus.* In addition, haematological parameters such as lymphocytes, white and red blood cells, haemoglobin and haematocrit increased significantly with the level of incorporation of *X. aethiopica*. On the other hand, MCV and MCH significantly decreased. In view of the above, X. *aethiopica* pod powder can be incorporated at 0.5% to improve reproductive performance of *C. gariepinus*.

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