

Full length Research paper

Effect of clove (*Eugenia aromatica*) powder anaesthetic on some haematological parameters in hybrid catfish (*Heterobranchus bidorsalis* ♀ x *C. gariepinus* ♂) juveniles

Okey B.^{1*}, Keremah R. I.² and Ofem B. O.¹

¹Cross River University of Science and Technology, Obubra Campus, Cross River State, Nigeria.

²Department of Fisheries/Livestock Production Technology, Niger Delta University, Amassoma, P. M. B. 071, Yenagoa, Bayelsa State, Nigeria.

Accepted 20 May 2013

This study was designed to investigate the efficacy of clove powder as anaesthetic and its effect on some haematological parameters of hybrid (*Heterobranchus bidorsalis* ♀ x *C. gariepinus* ♂) juveniles. Different concentrations of 80, 90, 100, 110, 120, and 130 mg/l were exposed to 108 healthy fish of mean weight 20.38 ± 3.30 g and total length 14.33 ± 2.50 cm for 30 min at the Fisheries Laboratory CRUTECH, Obubra campus. The fish behaviour was observed, the time to become completely immobilized and recovery from anaesthesia were recorded at the various stages with a stopwatch. Fish that were exposed to less than 100 mg/l clove powder reached Stage 3, but did not reach Stage 4 (complete immobilization) during the 30 min evaluation period. At the highest concentration of clove powder of 130 mg/l fish are anaesthetized (Stage 4) in less than 3 min and regained their normal position in more than 24 min. Fish exposed to higher concentrations above 100 mg/l took longer time to achieve recovery. The time to achieve recovery had a positive response to dose. The value of Red blood cell (RBC) shows a significant decrease while White blood cell count (WBC) with increase in the concentration of clove powder, then the haematological indices showed a significant increase in the values of (MCV) and Mean corpuscular Haemoglobin (MCH) while MCHC was not significantly different from the control ($p < 0.05$). The mean values of lymphocytes significantly increased whereas, neutrophil, monocytes, basophil, and eosinophil did not differ from the control. Result from this study suggests that, the use of clove powder at the concentration of 120 mg/l will completely induced deep anaesthesia without significant changes in the haematological parameters.

Key words: Clove powder, anaesthesia, recovery, haematological parameter, catfish hybrid.

INTRODUCTION

Anaesthetics play an important role in both fisheries research and aquaculture, being used to facilitate various handling procedures (Summerfelt and Smith, 1990; Siwicki, 1984). Anaesthetics act with various intensity

driving fish into general anaesthesia, resulting in loss of consciousness, inhibition of reflex activity and reduced skeletal muscle tone (Ross and Ross, 1990). Regardless of the agent, the process of anaesthesia in fish, develops

*Corresponding author. E-mail: piusbass@yahoo.com.

in a similar way and runs in a progressive pattern. Overdosing an anaesthetic or retaining the fish in an anaesthetic bath for too long leads to the fading of ventilation, hypoxia, finally respiratory and cardiac collapse (Tytler and Hawkins, 1981).

The fading of ventilation is an important warning sign suggesting that, the exposure should be terminated (Hajek and Kyszejko, 2004; Dziaman et al., 2005). Anaesthetics in fish farms are used to minimize motility during handling and transport. This may reduce susceptibility to pathogens and infection (Woody et al., 2002). Anaesthetics are also used in fish during artificial spawning, weighing, tagging, grading, and surgical procedures (Anderson et al., 1997). Wide range anesthetics are used by fish biologists to aid capture, handling and transport of fishes during practices that include enumeration, pathological analyses, hormonal implants, injections, vaccinations, stripping, transfer, and hauling (Carmichael and Tomasso, 1988; Brown, 1993). An ideal anaesthetic should induce anaesthesia rapidly with minimum hyperactivity or stress. It should be easy to administer and should maintain the animal in the chosen state.

When the animal is removed from the anaesthetics, recovery should be rapid. The anaesthetic should be effective at low doses and the toxic dose should greatly exceed the effective dose hence a wide margin of safety (Coyle et al., 2004). The properties addressed for choosing efficient anaesthetic agent for the fish will vary with objectives and species, but in general, fast induction (within 5 min), fast recovery (within 15 min), and no subjected mortality are among the most prevalent criteria considered in safe anaesthesia (Woody et al., 2002). Anesthetics most commonly used by commercial fish culturist and marine biologist during experiments in the field or laboratory includes quinaldine, quinaldine sulphate, tricaine methane sulphonate (MS-222), propanidid, benzocaine-hydrochloride, metomidate, chlorobutanol, phenoxyethanol, carbondioxide and clove oil (Marking and Meyer, 1985; Yanar and Kumlu, 2000; Velisek et al., 2005). Conventional anaesthetics such as tricaine methane sulphonate (MS-222), benzocaine and quinaldine are hazardous, expensive, not very effective and not readily available in some third world countries (Munday and Wilson, 1997).

Haematological indices are important parameters for the evaluation of fish physiological status. They are closely related to the response of the animal to the environment, an indication that, the environment where fishes live could exert some influence on the hematological characteristics (Gabriel et al., 2004). Their changes depend on the fish species, age, the cycle of sexual maturity and health condition (Wedemeyer et al., 1990; Luskova, 1997; Vosyliene, 1999). These indices have been employed in effective monitoring of the responses of fishes to stressors and thus, their health status under such adverse conditions. They can provide

substantial diagnostic information once reference values are established under standardized conditions. Evaluation of the haemogram involves the determination of the total erythrocyte count or Red blood cell (RBC), total White blood cell count (WBC), haematocrit or pack cell volume (PCV), Haemoglobin concentration (Hb), erythrocyte indices such as mean corpuscular volume (MCV), mean corpuscular Haemoglobin (MCH), Mean corpuscular haemoglobin concentration (MCHC), white blood cell differential count such as lymphocytes, neutrophil, basophil, eosinophil, monocytes and thrombocytes or platelet (Campbell, 2004).

Clove oil is considered to be a potential fish anaesthetic (Woody et al., 2002). According to Isaacs (1983), Briozzo et al. (1989) and Keene et al. (1998), the active ingredient of *Eugenia caryophyllata* are eugenol (4-allyl-2-methoxyphenol), which makes up 70 to 90% by weight of clove oil. Studies on the acute toxicity of clove products using values of haematological profile, biochemical profile and histological tissue examinations, to assess the effects on fishes exposure to anaesthetic have been investigated (Velisek et al., 2005a; 2005b). Recent studies have reported clove products as an effective anaesthetic for sedating fish for a number of invasive and non-invasive fisheries management and research procedures (Soto and Burhanuddin, 1995; Anderson et al., 1997; Keene et al., 1998; Prince and Powell, 2000; Srivastava et al., 2005).

More recently, efforts have been devoted to testing the efficacy of clove products for use in the fish culture industry (Taylor and Roberts, 1999; Wagner et al., 2002). Several studies have also compared the physiological effects of using clove products versus conventional anaesthetics. Clove products consistently yields similar levels of physiological disturbance and minimizes responses to external stressors to that observed with MS 222 (Cho and Heath, 2000; Sladky et al., 2001; Wagner et al., 2003). Collectively, the range of studies available suggests that, clove products are effective alternative for the sedation of fish. Soto and Burhanuddin (1995) first evaluated clove oil as an anaesthetic for Rabbitfish, *Siganus lineatus* curvier and valenciennes and found both rapid induction (mean, 108 s) and recovery (mean, 76 s) times at concentrations of 100 mg/l. Comparative efficacy trials on larval *Pomacentrus amboinensis* (Bleeker), a coral reef fish, indicated clove oil was more effective than MS -222, with more rapid induction and less variable response times (Munday and Wilson, 1997). Tests on juveniles and adults rainbow trout *Oncorhynchus mykiss* (Walbaum) indicate short exposures (<5 min) to clove oil concentrations of 40 and 120 mg/l will not affect subsequent swimming performance of either life stages (Anderson et al., 1997). Taylor and Roberts (1999) examined the efficacy of clove oil on juvenile and adult chinook salmon *Oncorhynchus tshawytscha* (Walbaum), juvenile coho salmon *Oncorhynchus kisutch* (Walbaum), juvenile rainbow trout

and juvenile and sub-adult white sturgeon *Acipenser transmontanus* Richardson. All fishes were safely immobilized and held up to 120 min in a concentration of 25 mg/l, however, median lethal concentrations (10 min exposures) ranged widely between the two genera (Chinook salmon 62 mg/l; white sturgeon 526 mg/l).

Prince and Powell (2000) evaluated that, clove oil concentration of 30 mg/l, was effective at inducing and maintaining deep anaesthesia for invasive surgery in adult rainbow trout. One potential drawback is that, recovery from clove oil anaesthesia can be up to 10 times longer than recovery from MS -222 (Anderson et al., 1997; Keene et al., 1998). Sudagara et al. (2009) reported that, 7 min exposure to 175, 225, 275, and 350 mg/l clove powder had a significant decreased in the haematocrit, hemoglobin, and total erythrocyte after anaesthesia on *Rutilus rutilus* (Roach). Isoeugenol (Clove oil) is currently recognized as the best anaesthetics for food fish by FDA due to its zero withdrawal time (fish can be consumed immediately after use). It is considered an alternative to other conventional anaesthetic such as MS -222 because of its short induction and longer recovery period, which is a useful trait for situation such as marking, surgery or spawning, where it is desirable to work with the fish for a longer period after been removed from the anaesthetics (Keene et al., 1998; Prince and Powell, 2000). Clove oil can serve as anaesthesia for a variety of fish; however, lengthy exposures can cause mortality and sub-acute morbidity to a variety of fish species.

In Nigeria, catfish hybrid is widely cultured and noted for their hardy, fast growth and commonly cultured in freshwater throughout the country. Research on the use of some indigenous plant materials for tranquilizing and anaesthetizing clariids and cichlids have been reported (Eze, 1991; Ayuba and Ofejukwu, 2004; Adebayo et al., 2010; Mgbenka and Ejiofor, 1998). No work on clove powder anaesthetic to catfish hybrid has been reported so far. The haematological characteristics of African mudfish, *C. buthupogon* have been reported (Kori-Siakpere and Egor, 1999). They have also been reports on the chronic sublethal haematological effects of copper in fresh water teleost, *Clarias isheriensis* and some alterations in haematological parameters in *C. isheriensis* exposed to sublethal concentrations of water borne lead (Kori-Siakpere 1991, 1995).

Clove powder is produced from the dry flower bud and flower stalk of the clove tree (*Eugenia spp*) and has been used for the short term immobilization of fish. The efficacy of clove powder has been evaluated by Sudagara et al. (2009) on the fish *R. rutilus* (Roach) in Iran and reported irreversible damage on the bloodstream. Clove powder is readily soluble in water unlike clove oil which needs to be dissolved first in alcohol or acetone before use. Clove flower buds readily available and very cheap used primarily as spice but with paucity of information on its usage as anaesthetics on

culturable species especially in Nigeria. The study of this kind will give baseline information on the use of clove powder as anaesthetics for Aquaculture development in Nigeria.

MATERIALS AND METHODS

Two hundred and fifty healthy hybrid (*Heterobranchus bidorsalis* ♀ x *C. gariepinus* ♂) juveniles with mean body weight 20.38 ± 3.30 g and total length of 14.33 ± 2.50 cm were procured from Unical fish farm, Calabar. They were transported in 3 separate 50 L jerrycans by car to the Fisheries Laboratory, CRUTECH, Obubra Campus. The fish were acclimated for one week in groups of 10 fish per rectangular glass aquaria with 30 L of borehole water. The water in the aquaria was renewed daily and they were fed twice a day with a commercial feed (coppen) at 1% body weight at 0800 and 1600 h, respectively. Feeding was discontinued 24 h before the commencement and during the experiment to minimize the contamination of the test media. No mortality was recorded during the acclimation period. Dry *E. caryophyllata* flower buds were procured from a herbal shop in watt market Calabar, Calabar South Local Government Area of Cross River State, Nigeria. The materials were identified at the Department of Botany University of Calabar. They were taken to the Fisheries Laboratory Cross River University of Technology (CRUTECH,) Obubra Campus. The flower buds were sundried for 30 min and then pulverized with a sterile manual blender and sieved with 100 micron net to obtain a fine powder. The powder was put in an airtight container and stored in a dry place.

A stock solution of clove power with a concentration of 200 mg/L was prepared by dissolved 2 g of *E. aromaticum* powder into 10 L of borehole water. A range finding test was conducted to determine induction time (Anaesthesia) of clove power following the method of (Bell, 1987; King et al., 2005). The range finding concentrations for the induction (anaesthesia) were 50, 60, 80, 100, 120, and 140 mg/l in 20 L of water obtained from serial dilution of the stock solution. Exposure concentrations of clove powder were 80, 90, 100, 110, 120 and 130 mg/l, respectively.

Thirty six glass aquaria were clean and randomly labeled and each filled with water to the 15 liters mark for induction test and 20 L mark for recovery. The different concentrations were prepared by serial dilution of the stock solution and water added to make up to 20 L that gave the desired concentrations. The mixture was stirred with a glass rod for homogenous mixing. Each concentration was stock with 6 juveniles in triplicate and monitored for the on set of induction (anaesthesia) for 30 min as periods greater than this were considered impractical for routine fish handling procedures.

Any of the test fish that lost balance and ceased respiratory movements of the opercula (deep anaesthesia) was removed immediately and transferred to about 20 L of clove powder free water. The time of induction to the clove powder and the recovery time were noted with a stopwatch. The induction time was defined as the time taken from the moment the fish was exposed to the anesthetic to the moment the respiratory movement of the opercula stopped. Recovery time was defined as the time taken from the moment the fish was considered anaesthetized until the moment regular respiratory movements were resumed. None of the revived fish were re-used for further experimentation but were kept in another glass aquaria and plastic buckets.

On determining the safest and most effective concentration, the experiment was repeated in triplicates using batches of 6 randomly selected fish each time. The time for the fish to enter the desirable anaesthesia level (induction) that which is needed for an anesthetized fish to regain equilibrium and began active swimming (recovery time) were recorded at each stages (Table 1). Water quality parameters such, temperature, $26.71 \pm 1.92^\circ\text{C}$; dissolved

Table 1. Stages of anaesthesia (Induction) and recovery.

Anaesthesia (induction)		Recovery	
Stage	Behavior of the fish	Stage	Behavior of the fish
1	Acceleration of the opercular movements, increased respiratory activity, accompanied by uncoordinated locomotion.	1	Body immobilized but opercular movements just starting and weak, uncoordinated locomotion
2	Sporadic loss of equilibrium, difficulty maintaining position while at rest, high reaction to external stimuli.	2	Regular opercular movements and gross body movements beginning.
3	Complete loss of equilibrium; inability to regain upright position.	3	Equilibrium regained, normal swimming and pre-anaesthetic appearance.
4	No reaction to handling or a sharp prod in the peduncle.		

From Iwama et al. (1989).

Table 2. Time for induction and recovery from anesthesia of *Clarias gariepinus* exposed different concentrations of Clove powder for 30 min.

Conc. (mg/l)	Stages of induction time (min)				Stages of recovery (min)		
	I	II	III	IV	I	II	III
80	11.67 ± 0.67 ^a	22.87 ± 0.20 ^a	-	-	-	-	-
90	7.53 ± 0.24 ^b	14.07 ± 30 ^b	20.93 ± 0.41 ^a	-	-	-	-
100	2.60 ± 0.12 ^c	4.86 ± 2.9 ^c	8.26 ± 0.41 ^b	22.32 ± 0.22 ^a	1.66 ± 0.19 ^c	3.43 ± 0.18 ^d	4.55 ± 0.25 ^d
110	1.93 ± 0.18 ^d	2.93 ± 0.35 ^d	5.40 ± 0.31 ^c	13.40 ± 0.80 ^b	2.00 ± 0.19 ^c	6.89 ± 0.73 ^c	10.45 ± 0.22 ^c
120	-	1.80 ± 0.23 ^e	2.87 ± 0.13 ^d	4.07 ± 0.13 ^c	3.29 ± 0.19 ^b	8.56 ± 0.88 ^b	17.00 ± 0.51 ^b
130	-	-	1.20 ± 0.12 ^e	2.60 ± 0.12 ^d	5.67 ± 0.38 ^a	11.76 ± 0.63 ^a	24.48 ± 0.10 ^a

Mean with the same superscript are not significantly different at $p < 0.05$.

oxygen, 4.7 ± 1.37 mg/l; pH, 7.12 ± 1.21 ; total alkalinity, 18.3 ± 2.32 mg/l and total hardness, as CaCO_3 , 17.77 ± 1.44 mg/l were monitored and recorded.

Immediately after 30 min of induction (anaesthesia), 3 fish each per aquaria were sample and mopped with tissue paper to prevent haemolysis due to dilution of oozing blood with any other body fluid. Blood of 5 ml were collected by severing the caudal peduncle of the fish into heparinized plastic tubes containing the sodium salt of Ethylene Diamine Tetra Acetic acid (Na-EDTA) as an anticoagulant. The samples were preserved in a cooler containing ice block and transported to Biochemical Laboratory, Department of Biochemistry Unical with in 6 h for haematological analyses. RBC, total white blood cell WBC and platelet counts were done using the Neubauer haemocytometer. The haematocrit or packed cell volume (PCV) and haemoglobin (Hb) concentration values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods (Hesser, 1960), respectively. The MCV, MCH, and MCHC were calculated from the data using standard formulae (Lee et al., 1998).

Data obtain from the various stages of induction (anaesthesia), recovery and from blood analyses were subjected to various statistical tools. Descriptive statistic was used to obtain the means and standard error (Mean ± S.E). Differences among time for different dosage to achieve various stages of anesthesia, recovery time and blood parameters on concentration were subjected to one way ANOVA using SPSS 15.0 version. Duncan's multiple range

tests was used to compare the various means at ($P < 0.05$) significance level. Pearson's correlation was also used to check the relationship among the various haematological indices according to Zar (1999).

RESULT

Induction (anaesthesia) and recovery

The result of the various time required for induction and recovery from anaesthesia using clove powder in catfish hybrid juveniles for 30 min exposure time is shown in Table 2. Fish that were exposed to less than 100 mg /l clove powder reached Stage 3, but did not reach Stage 4 (complete immobilization) during the 30 min evaluation period. Fish exposed to 100 mg/l were completely immobilized at less than 23 min immediately after transferring them into the recovery tanks normal locomotor activity was observed with 1.5 to 11 min. The increasing concentration of clove powder proportionally decreased the time required for sedation and anesthesia

Table 3. Effect of clove powder anaesthetic on haematological indices in catfish hybrid juveniles.

Conc. (mg/l)	Haematological indices						
	PCV (%)	RBCC ($10^6/\text{mm}^3$)	Hb (g/l)	Plt ($10^3/\text{mm}^3$)	MCV (fl)	MCH (pg)	MCHC (g/l)
0.00	37.47 $\pm$ 0.56 ^a	6.13 $\pm$ 0.69 ^a	9.23 $\pm$ 0.43 ^a	55.53 $\pm$ 6.67 ^d	59.87 $\pm$ 6.56 ^d	15.50 $\pm$ 2.06 ^d	25.77 $\pm$ 0.14 ^b
80	34.77 $\pm$ 2.16 ^{ab}	5.10 $\pm$ 0.57 ^{ab}	8.80 $\pm$ 0.11 ^{ab}	66.00 $\pm$ 4.28 ^{cd}	72.83 $\pm$ 5.79 ^c	18.40 $\pm$ 2.19 ^c	24.15 $\pm$ 0.64 ^b
90	33.60 $\pm$ 1.89 ^{abc}	4.13 $\pm$ 0.49 ^b	7.83 $\pm$ 0.44 ^{bc}	79.78 $\pm$ 5.36 ^c	82.62 $\pm$ 6.98 ^{abc}	19.27 $\pm$ 1.49 ^c	23.45 $\pm$ 1.34 ^b
100	33.37 $\pm$ 2.69 ^{abc}	3.70 $\pm$ 0.49 ^{bc}	8.03 $\pm$ 0.29 ^{bc}	79.70 $\pm$ 7.48 ^c	91.93 $\pm$ 4.98 ^{abc}	22.36 $\pm$ 1.98 ^b	24.29 $\pm$ 1.39 ^b
110	29.77 $\pm$ 1.99 ^{bcd}	3.88 $\pm$ 4.68 ^{bc}	7.97 $\pm$ 0.68 ^c	104.63 $\pm$ 4.85 ^b	97.77 $\pm$ 7.65 ^{ab}	20.48 $\pm$ 6.19 ^{bc}	26.17 $\pm$ 2.98 ^{ab}
120	28.65 $\pm$ 0.89 ^{cd}	3.40 $\pm$ 0.56 ^{bc}	7.63 $\pm$ 0.56 ^c	119.43 $\pm$ 2.68 ^a	99.76 $\pm$ 9.87 ^{ab}	26.17 $\pm$ 2.98 ^a	24.28 $\pm$ 0.99 ^b
130	26.48 $\pm$ 1.29 ^d	2.20 $\pm$ 0.72 ^c	6.27 $\pm$ 0.26 ^d	129.28 $\pm$ 3.87 ^a	105.06 $\pm$ 3.99 ^a	28.76 $\pm$ 1.29 ^a	29.88 $\pm$ 0.88 ^a

Mean with the same superscript are not significantly different at $p < 0.05$, PCV = Packed cell volume, RBCC = Red blood cell counts Hb = Haemoglobin, MCV = Mean cell volume MCH = Mean cell haemoglobin MCHC = Mean haemoglobin concentration.

Table 4. Effect of clove powder anaesthesia on differential white blood count in catfish hybrid juveniles.

Conc. (mg/l)	Differential white blood cell count ($10^3/\text{mm}^3$)					
	WBCC	Neut	Lymp	Baso	Mono	Eosin
0.00	62.97 $\pm$ 2.99 ^d	8.65 $\pm$ 0.29 ^a	13.66 $\pm$ 0.39 ^d	2.89 $\pm$ 0.11 ^a	1.97 $\pm$ 0.28 ^a	7.39 $\pm$ 0.59 ^a
80	67.43 $\pm$ 1.81 ^d	8.93 $\pm$ 0.29 ^a	14.08 $\pm$ 0.60 ^{cd}	2.83 $\pm$ 0.13 ^a	1.83 $\pm$ 0.14 ^a	7.89 $\pm$ 0.59 ^a
90	72.50 $\pm$ 3.19 ^{cd}	7.95 $\pm$ 0.14 ^b	14.33 $\pm$ 0.15 ^{cd}	2.75 $\pm$ 0.23 ^a	1.87 $\pm$ 0.18 ^a	7.46 $\pm$ 0.47 ^a
100	86.17 $\pm$ 6.88 ^c	7.88 $\pm$ 0.13 ^b	15.20 $\pm$ 0.50 ^c	2.09 $\pm$ 0.08 ^{bc}	1.76 $\pm$ 0.28 ^a	7.30 $\pm$ 0.13 ^a
110	101.03 $\pm$ 6.84 ^b	7.64 $\pm$ 0.06 ^{bc}	15.40 $\pm$ 0.47 ^c	2.40 $\pm$ 0.20 ^b	1.59 $\pm$ 0.39 ^a	7.93 $\pm$ 0.07 ^a
120	115.17 $\pm$ 7.98 ^b	7.60 $\pm$ 0.17 ^{bc}	16.83 $\pm$ 0.29 ^b	1.81 $\pm$ 0.74 ^c	1.89 $\pm$ 0.22 ^a	6.93 $\pm$ 0.09 ^b
130	135.97 $\pm$ 3.77 ^a	7.14 $\pm$ 0.16 ^c	18.69 $\pm$ 0.75 ^a	1.87 $\pm$ 0.17 ^c	1.56 $\pm$ 0.35 ^a	6.69 $\pm$ 0.10 ^b

Mean with the same superscript are not significantly different at $p < 0.05$, Neut = neutrophil Lymp= lymphocytes Baso = basophil, Mono = monophil, Eosino = eosinophil, WBCC = white blood cell count.

(induction). As the induction was very rapid, it was not possible to visualize Stage 2 in fish that were exposed to concentrations of 130 mg/l. No mortality resulted from anesthesia induction within the range of 80 and 130 mg/l. The time of recovery was significantly faster following exposure to the lowest concentrations (100 and 130 mg/l). Catfish hybrid juveniles that were exposed to low concentrations (80 and 90 mg/l) of the clove powder for 30 min maintained a uniform depth of sedation, that is, they remained at Stages 2 or 3.

Fish exposed to higher concentrations took longer time to achieve recovery. The time to achieved recovery had a positive response to dose. Fish exposed to 130 mg/l took less than 4 min to be anaesthesia and more 24 min to recover fully. At concentrations of 120 mg/l fish reach Stage 4 within 5 min and the recovery and returning to normal swimming position was achieved with in 11 to 22 min. At the highest concentration of clove powder of 130 mg/l fish are anaesthetized (Phase 4) in less than 3 min and they regained their normal position in more than 24 min. In any case, a quick induction and a recovery time

which allows for varied manipulations are desirable.

In addition, anaesthetics should be safe, easy to handle and cheap more in a developing country like Nigeria. The present observation suggests that, clove powder extracts acted as an anaesthetic in the catfish juveniles. The sequential progression through the various stages of anesthesia with increasing dose and time and the recovery of anaesthetized fish, all followed the patterns of typical fish anaesthetic (Marking and Meyer, 1985).

Haematological responses

The result on the effects of clove powder anaesthetic on haematological and differential white blood cell counts of catfish hybrid is shown in Tables 3 and 4. The values were compared with the corresponding values in the control. The mean values of the RBC, Hb, and PCV, decrease with increase in concentration of clove powder. The mean values of the PCV and that of the control were not significantly different at 80 to 100 mg/l, but were

different at 110 to 130 mg/l.

However, the values of WBC, platelet and erythrocytes indices (MCV, MCH and MCHC) increase with increasing concentration of clove powder. The mean value of Hb in the control was 9.04 g/l and the lowest concentration (80 mg/l) was 8.83 g/l and was not significantly different and ($P < 0.05$). Lower values of neutrophils, monocytes, eosinophils, and basophils were obtained in the control group compared with the group exposed to clove powder. The mean values of lymphocytes increased with increased in concentration; with the lowest value obtained in fish exposed to 80 mg/l and the highest in 130 mg/l of clove powder. Monocytes and eosinophil were not significantly different from those of control at ($P < 0.05$), the value of lymphocytes increased while basophil decreased significantly from those of control ($P < 0.05$). The mean values of lymphocytes significantly increased whereas, neutrophil, monocytes, basophil and eosinophil did not differ from the control at ($P < 0.05$) as shown in Table 4.

The ANOVA shows that clove powder anesthetics caused a significant ($P < 0.01$) decrease in the PCV, RBC and Hb whereas, WBC and platelet were significantly increase at ($P < 0.001$) compared with the control (Table 5). Differential leucocytes values shows that, neutrophil, lymphocytes and basophil were significant different from those of control $P < 0.05$ (Table 6).

DISCUSSION

According to the tests with juveniles of *C. gariepinus*, the concentration of the clove powder that is required to induce deep anesthesia (Stage 4) is 100 mg/l or higher. However, to obtain rapid anesthesia (< 4 min), concentrations of 120 to 130 mg/l must be used. The recovery time of anesthesia induced by this powder was 5 to 24 min. Hikasa et al. (1986) recommended 25 to 100 ppm clove oil as effective anaesthesia for the common carp (*Cyprinus carpio*). It has been demonstrated that, onset times of individual stages of clove powder anaesthesia as well as recovery times were concentration-dependant.

The same effect of anaesthetic concentration levels on anaesthesia onset times has been described by Hirata et al. (1970) for the crucian carp (*Carassius carassius*), Hamackova et al. (2001) for the tench (*Tinca tinca*) and Zaikov et al. (2008). In this study, effective concentration was between 120 to 130 mg/l. Waterstrat (1999) reported 100 mg/l clove oil as a safe concentration for anaesthesia of the channel catfish (*Ictalurus punctatus*), adding that exposures longer than 15 min prolonged recovery times and increased mortality. Walsh and Pease (2002) recommended 60 to 80 mg/l clove oil for anaesthesia of anguillid eels (*Anguilla reinhardtii*) because it is effective, relatively inexpensive, and poses

little risk to human health. The main focus of this study was on the time needed for the juveniles to reach the various stages of (3 and 4) anaesthesia and the time needed for the recovery. Fish exposed to 100mg/l were completely immobilized at less than 23 min while those exposed to 130 mg/l took less than 4 min to be anaesthesia. The observed progression through these various stages of anaesthesia was consistent with the descriptions by Keen et al. (1998), Zaikov et al. (2008) and Hikasa et al. (1986). It has been demonstrated that, onset times of individual stage of clove powder anesthesia as well as recovery follows the same pattern has those described by Hirata et al. (1970) for the crucian carp (*Carassius carassius*), Hamackova et al. (2001) for Tench (*Tinca tinca*), Grush et al. (2004) for Zebra fish (*Danio rerio*) and Velisek et al. (2005) for common carp (*C. carpio*).

Haematological indices are closely related to response of the animal to the environment (Fernandes and Manson, 2003). Ralio et al. (1985) reported that, the blood parameters of diagnostic importance are erythrocytes counts, leucocytes counts, Hb, haematocrits and differential leucocytes counts, because they readily respond to physical and environmental stress due to water contaminants. Previous study shows that, clove oil anaesthesia 30 mg/l concentration and 10 min exposure had no effects on the hematological indices of *C. carpio* and *Oncorhynchus mykiss* (Velisek et al., 2005a, 2005b). Researchers have reported a significant decrease in PCV, Hb, and erythrocytes, while the leucocytes increased when *C. gariepinus* is exposed to various pollutants under laboratory conditions (Adeyemo, 2005; Aderolu et al., 2010; Okomoda et al., 2010).

In this study, the significant decrease ($P < 0.05$), of haematocrit value, Hb concentrations and RBC after 30 min clove powder anaesthesia was observed and their changes were concentration dependent. This agrees with the findings of Adamek et al. (1993) and Velisek et al. (2005a) who reported decreased RBC, Hb concentration and hematocrit values in *C. carpio* following 2-phenoxyethanol (0.30 ml/l) anaesthesia. Sudagara et al. (2009) reported that, 7 min exposure to 175, 225, 275 and 350 mg/l clove powder had a significant decrease in the haematocrit, hemoglobin and total erythrocyte after anaesthesia on *R. rutilus* (Roach). Haematology studies in teleosts have indicated that, haematocrit values might be useful as a general indicator of fish health, since fish given iron deficient diets, or those exhibiting anaemia; all possess reduced haematocrit (PCV) values (Gatlin and Wilson, 1986).

However, Farathi et al. (2011) and Imanpoor et al. (2010) both reported a significant increase in PCV, RBC, and Hb while the WBC were significantly decreased when clove essence exposed to Prussian carp and *Acipenser persicus*, respectively. Their findings disagree with that of this research. In general, physiological responses in fish to anaesthetics are

Table 5. One way ANOVA for the haematological parameters catfish hybrid exposed to clove powder anaesthetic.

Parameter	Source of variation	Sum of squares	df	Mean square	F	Significant
PCV	Treatments	265.460	6	44.243	5.116	**
	Error	121.064	14	8.647		
	Total	386.524	20			
RBC	Treatments	28.338	6	4.723	5.110	**
	Error	12.940	14	.924		
	Total	41.278	20			
Hb	Treatments	16.029	6	2.671	9.690	***
	Error	3.860	14	.276		
	Total	19.889	20			
WBC	Treatments	13233.010	6	2205.502	26.748	***
	Error	1154.360	14	82.454		
	Total	14387.370	20			
Plt	Treatments	13783.219	6	2297.203	28.651	***
	Error	1122.513	14	80.180		
	Total	14905.732	20			
MCV	Treatments	4498.315	6	749.719	3.960	.
	Error	2650.232	14	189.302		
	Total	7148.547	20			
MCH	Treatments	379.934	6	63.322	4.971	.
	Error	178.350	14	12.739		
	Total	558.284	20			
MCHC	Treatments	88.446	6	14.741	3.618	*
	Error	57.037	14	4.074		
	Total	145.483	20			

F-test significance level: ns-non-significant, *- 0. 05, **- 0.01, ***- .001.

Table 6. One way ANOVA for the differential white blood cell count of catfish hybrids exposed to clove powder anaesthetic.

parameter	Source of variation	Sum of squares	df	Mean square	F	Significant
Neutro	Treatments	7.068	6	1.178	11.262	***
	Error	1.464	14	0.105		
	Total	8.533	20			
Lymp	Treatments	57.204	6	9.534	14.759	***
	Error	9.043	14	0.646		
	Total	66.247	20			
Monop	Treatments	.486	6	0.081	0.511	Ns
	Error	2.220	14	0.159		
	Total	2.706	20			
Eosinop	Treatments	2.657	6	0.443	1.194	Ns
	Error	5.193	14	0.371		
	Total	7.850	20			
Basop	Treatments	3.818	6	0.636	14.581	***
	Error	.611	14	0.044		
	Total	4.429	20			

F-test significance level: ns-non-significant, *- 0. 05, **- 0.01, ***- .001.

different. The result from this study indicated that, clove powder at the concentrations of 110 to 130 mg/l induced

deep anaesthesia between 3 to 10 min with a recovery time of between 8 to 24 min making it an ideal

anaesthetic for catfish hybrid juveniles.

Conclusion

The use of clove powder as an anaesthetic for catfish hybrid is a better alternative as it can be safely utilized on them and recovery times are shorter than with other conventional anaesthetics. Given that, there is a high correlation between the anaesthetic dosage and recovery time, smaller dose of the anaesthetic could be utilized in inducing sedation for longer period, thus, making clove powder a highly effective, readily available, highly soluble and cheap fish anaesthetic with potentially little effect on some haematological parameters which have been reported reversible.

REFERENCES

- Adamek Z, Fasaic, K, Paul A, Lamesic M (1993). The effect of 2-phenoxyethanol necrosis on the blood parametry of young carp (*Cyprinus carpio*). Vet. Arch. 63:245-250.
- Adebayo TO, Fasakin EA, Popoola OM (2010). Use of Aqueous Extracts of Avocado Pear, *Pyrus Communis*, Leaf as Anaesthetic in Gonadectomy of African Catfish, *Clarias gariepinus*. J. Appl. Aquacul. 117-122.
- Aderolu AZ, Ayoola SO, Otitoloju AA (2010). Effects of Acute and sub-lethal concentrations of Actellic on Weight changes and Haematology parameters of *clarias gariepinus*. World J. Biol. Res. 3:30-39.
- Adeyemo OK (2005). Haematological and histopathological effects of cassava mill Effluent in *Clarias gariepinus*. Afr. J. Biomed. Res. 8:179-183.
- Anderson GW, McKinley SR, Colavecchia M (1997). The use of clove oil as an anaesthetic for rainbow trout and its effects on swimming performance. North Am. J. Fish. Manage. 17:301-307.
- Ayuba VO, Ofojekwu PC (2002). Acute toxicity of the root extract of Jimson's weeds *Datura innoxia* to the African catfish, *C. gariepinus* fingerlings. J. Aquac. Sci. 17(2):131-136.
- Bell G (1987). An outline of anesthetic and anesthesia for salmonids, a guide for fish culturists in British Columbia. Can. Tech. Rep. Fish. Aquat. Sci. 1534:16.
- Briozzo J, Nunez L, Chirife J, Herszage L, D'Aquino M (1989). Antimicrobial activity of clove oil dispersed in a concentrated sugar solution. J. Appl. Bacteriol. 66:69-75.
- Brown LA (1993). Anaesthesia and restraint. In: Stoskopf, M.K. (Ed.), Fish Medicine. WB Saunders, Philadelphia. pp. 79-90.
- Carmichael GJ, Tomasso JR (1988). Survey of fish transportation and techniques. Prog. Fish-Cult. 50:155-159.
- Cho GK, Heath DD (2000). Comparison of tricaine methane sulphonate (MS222) and clove oil anaesthesia effects on the physiology of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum). Aquac. Res. 31:537-546.
- Coyle SD, Durborow RM, Tidwell JH (2004). Anaesthetics in Aquaculture. SRAC publication, Texas. P. 6.
- Dziaman R, Kyszejko B, Hajek G (2005). The effects of MS-222 on the cardiac and respiratory function and behaviour of common carp, *Cyprinus carpio* L., during general anaesthesia. Acta Ichthyologica et Piscatoria 35:125-131.
- Eze CC (1991). Tranquilizing and anaesthetizing effects of some indigenous plants in fish aquaculture. Bachelor Thesis. University of Nigeria, Nsukka Nigeria.
- Farathi A, Kasiri M, Sudagar M, Iraei MS (2011). Size-Relative Effectiveness of Clove Essence as an Anesthetic for Kutum (*Rutilus frisii kutum*) Global Veterinaria 6(2):180-184, ISSN 1992-6197.
- Fernandes MN, Mazon AF (2003). Environmental pollution and fish gill morphology, In: Fish Adaptions. (Val AL, Kapoor BG eds.). Sci. Pub. Enfield, USA. pp. 203-231.
- Gabriel UU, Ezeri GNO, Opabunmi OO (2004). Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). Afr. J. Biotech. 3(9):463-467.
- Hajek GJ, Kyszejko B (2004). The effects of Propiscin (etomidate) on the behaviour, heart rate, and ventilation of common carp, *Cyprinus carpio* L. Acta Ichthyologica et Piscatoria 34:129-143.
- Hamackova J, Sedova JM, Pjanova SV, Lepicova A (2001). The effect 2-phenoxyethanol, clove oil and Propiscin anaesthetics on perch (*Perca fluviatilis*) in relation to water temperature. Czech J. Anim. Sci. 46:469-473.
- Hikasa Y, Katsuaki T, Ogasawara T, Ogawara S (1986). Anesthesia and recovery with tricaine methanesulfonate, eugenol, and thiopental sodium in the carp, *Cyprinus carpio*. Jpn. J. Vet. Res. 48:341-351.
- Hirata M, Isoda S, Kanao M, Shimizu H, Inouse S (1970). Studies on anesthetics for fish. Bulletin of Jap-anese Society of Science Fisheries, 36:1127-1135.
- Imanpoor MR, Bagheri T, Hedayati SAA (2010). The Anesthetic Effects of Clove Essence in Persian Sturgeon (*Acipenser persicus*). World J. Fish Marine Sci. 2(1):29-36.
- Isaacs G (1983). Permanent local anaesthesia and anhydrosis after clove oil spillage. Lancet 1:882-883.
- Iwama GK, McGeer JC, Pawluk MP (1989). The effects of five fish anaesthetics on acid-base balance, hematocrit, cortisol and adrenaline in rainbow trout. Can. J. Zool. 67:2065-2073.
- Keene JL, Noakes DLG, Moccia RD, Soto CG (1998). The efficacy of clove oil as an anesthetic for rainbow trout *Oncorhynchus mykiss*. Aquacult. Res. 29:89-101.
- King W, Hooper B, Hillsgrrove S, Benton C, Berlinsky D (2005). The use of clove oil, metomidate, tricaine methanesulphonate and 2-phenoxyethanol for inducing anaesthesia and their effect on the cortisol stress response in black sea bass (*Centropristis striata* L.). Aquac. Res. 36:1442-1449.
- Luskova V (1997). Annual cycles and normal values of hematological parameters in fishes. Acta. Sci. Nat. Brno. 31(5):70-78.
- Marking LL, Meyer FP (1985). Are better anaesthetics needed in fisheries? Fisheries 10:2-5.
- Mgbenka BO, Ejirofor EN (1998). Effect of extract of dried leaves of *Erythrophleum suaveolens* as anaesthetics on Clariid Catfish. J. Appl. Aquacult. 8(4):73-80.
- Munday PL, Wilson SK (1997). Comparative efficacy of clove oil and other chemicals in anaesthetisation of *Pomacentrus amboinensis*, a coral reef fish. J. Fish Biol. 51:931-938.
- Okomoda J, Ayuba VO, Omeji S (2010). Heamatological Changes of *Clarias gariepinus* (Burchell, 1822) Fingerlings Exposed To Acute Toxicity of Formalin. PAT 6(1):92-101 ISSN: 0794-5213. www.patnsukjournal.net/currentissue.
- Ralio E, Mikiñman M (1985). Effect of sampling on blood parameters in rainbow trout *Salmo gaidneri*. J. Fish. Res. Biod. 26:725-732.
- Ross B, Ross LB (1990). Anaesthetic and Seductive for Aquatic Animals. 2nd Edition, Blackwell Science Ltd.
- Siwicki A (1984). New anesthetic for fish. Aquaculture 38(2):171-176.
- Sladky KK, Swanson CR, Stoskopf MK, Loomis MR, Lewbart GA (2001). Comparative efficacy of tricaine methanosulfonate and clove oil for use as anaesthetics in red pacu (*Piaractus brachipomus*). Am. J. Vet. Res. 62:337-342.
- Soto CG, Burhanuddin (1995). Clove oil as a fish anaesthetic for measuring length and weight of rabbit fish (*Siganus lineatus*). Aquaculture 136:149-152.
- Srivastava AK, Srivastava SK, Syamsundar KV (2005). Bud and leaf essential oil composition of *Syzygium aromaticum* from India and Madagascar. Flavour Fragr. J. 20:51-53.
- Sudagara M, Mohammadizarejabada A, Mazandarania R, Pooralimotlagha S (2009). The efficacy of clove powder as an anesthetic and its effect on hematological parameters on Roach (*Rutilus rutilus*). J. Aquacult. Feed Sci. Nutr. 1(1):1-5, ISSN: 2070-1667.
- Summerfelt RC, Smith LS (1990). Anaesthesia, surgery, and related techniques. In: Schreck, C.B., Moyle, P.B. (Eds.), Methods for Fish Biology. American Fisheries Society, Bethesda, MD pp. 213-272.
- Taylor PW, Roberts SD (1999). Clove oil: an alternative anaesthetic for aquaculture. North Am. J. Aquacult. 61:150-155.
- Tytler R, Hawkins AD (1981). Vivisection, anaesthetics and minor

- surgery. In Aquarium (A.D. Hawkins, eds.), Academic Press London. pp. 247-278.
- Velisek J, Svobodova Z, Piackova V, Groch I, Nepejchalova I (2005a). Effects of clove oil anaesthesia on common carp (*Cyprinus carpio* L.). Vet. Med. – Czech. 50(6):269-275.
- Velísek J, Svobodová Z, Piačková V (2005b). Effects of Clove Oil Anaesthesia on Rainbow Trout (*Oncorhynchus mykiss*). Acta Vet. Brno. 74:139-146.
- Vosyliene MZ (1999). The effects of heavy metals on haematological indices of fish. Acta Zoologica Lituanica. Hydrobiologia 9(2):76-82.
- Wagner E, Arudt R, Hilton B (2002). Physiological stress responses, egg survival and sperm mobility for rainbow trout broodstock anesthetized with clove oil, tricaine methane sulfonate or carbon dioxide. Aquaculture 211:353-366.
- Wagner GN, Singer TD, McKinley RS (2003). The ability of clove oil and MS-222 to minimise handling stress in rainbow trout (*Oncorhynchus mykiss* Walbaum). Aquac. Res. 34:1139-1146
- Walsh CT, Pease BC (2002). The use of clove oil (eugenol), oil as an anaesthetic for the longfinned eel, (*Anguilla reinhardtii* Steindachner). Aquac. Res. 33:627-635.
- Waterstrat PR (1999). Induction and recovery from anesthesia in channel catfish (*Ictalurus punctatus*) fingerlings exposed to clove oil. J. World Aquacult. Soc. 30:250-255.
- Wedemeyer GA, Barton BA, McLeay DJ (1990). Stress and acclimation. In: Schreck CB, Moyle PB, eds. Methods for Fish Biology. Bethesda: Am. Fish. Soc. pp. 451-489.
- Woody CA, Nelson J, Ramstad K (2002). Clove oil as anesthetic for adult sockeye salmon: field trials. J. Fish Biol. 60:340-347.
- Yanar M, Kumlu M (2000). The Anaesthetic Effects of Quinaldine Sulphate and/or Diazepam on Sea Bass (*Dicentrarchus labrax*) Juveniles. Turk. J. Vet. Anim. Sci. 25:185-189.
- Zaikov A, Ilielv F, Hubenova TI (2008). Induction and recovery from anaesthesia in pike (*Esox lucius* L.) exposed to clove oil. Bulgarian J. Agric. Sci. 14(2):165-170
- Zar JH (1999). Biostatistical Analysis. 4th Edition Prentice Hall. New Jersey.