http://dx.doi.org/10.4314/bajopas.v10i2.24

Bayero Journal of Pure and Applied Sciences, 10(2): 141 - 145
Received: November, 2017
Accepted: December, 2017
ISSN 2006 – 6996

EFFECTS OF ORGANIC MANURE ON SOME HAEMATOLOGICAL PARAMETERS OF *Oreochromis niloticus, Clarias gariepinus* and *Cyprinus capio* IN POLYCULTURE SYSTEM

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ABSTRACT
The impact of poultry dropping, cow dung and rumen content on the haematology of Oreochromis niloticus, Clarias gariepinus and Cyprinus capio in a polyculture system were studied. The work was conducted over a period of six months. Poultry droppings (PD), cow dung (CD) and rumen content (RC) were applied at three levels 30g, 60g & 120g/m^2/week, 25g, 50g & 100g/m^2/week and 22g, 44g & 88g/m^2/week treatment respectively. While in the control only conventional feeds 40% Crude Protein was fed without manure application. Hemoglobin percentage decreases from 7.07 to 12.63% with a significant difference between fish species, There are no significant differences (P < 0.05) in Red Blood Cell count and haemolysis between treatments on the fish species. It can be concluded that higher levels of manure 120,100 and 88 g/m^2/week had no affect haematological parameters. The study revealed that increase and decrease of various haematology of test fish samples explains the good conditions caused by the manure in the pond water. Therefore, there is need to fertilize pond with organic manure for the benefit of increase in nutrients to nourish diverse fish species and the condition of the pond ecosystems.
Key words: Erythrocyte Fragility, Haemoglobin, Organic manure, Red Blood Cell,

INTRODUCTION

The hematological information of African freshwater fish species is very scanty when compared to reports on marine environment. Hematological parameters are used as a tool for assessing the health status of fish. Changes in blood parameters are quick responses to environmental or physiological alterations, and can provide an integrated measure of the physiological status of the organism (Annune *et al.,* 1994)

Freshwater fishes are often subjected to pollution, especially near industrial or populated areas. Organic materials and heavy metals have been known to exert a wide range of effects on fishes, from metabolic, physiological to behavioral and ecological factors (Forstner and Wittmann, 1981). These effects include disturbance in osmoregulation and respiration (Ellies, 1981), tissue damage (Annune *et.al.,* 1994), reduce energetic resources and poor performance (Health, 1984).

Hemoglobin levels changes with seasons, increasing during the cold seasons, other water qualities conditions such as oxygen and carbon dioxide tensions could cause a decrease of red blood cell volume by 25% and cause lymphopaenia (Belova, 1966; Conroy, 1972). Nitrates, element released from organic material intermediate oxidation product of ammonia and nitrate is more toxic to fish in freshwater compared with seawater (Grossel & Jensen, 2000; Jensen, 2003). The uptake toxicity mechanism and physiology effect of nitrate has been extensively studied in many fishes (Lewis and Morris 1986, Jensen *et al*., 1987; Stomer *et.al*., 1996; Knudsen and Jensen 1997; Vedel *et.al*., 1998; Grossel and Jensen 2000; Huang and Chen 2002; Jensen 2003). Freshwater fish take up NO_2 primarily across the gill, which affects tissues and the immune system of the body. Their responses are very similar to the effects of bio-accumulation of a pollutant, not only in the plasma, but also in the gill, liver, brain, spleen and muscle (Margiocco, *et.al*., 1983).

The indiscriminate use of manures in fish ponds instead of improving the pond productivity may lead to pollution. Therefore, it is imperative to know the standard doses of these organic manures which would keep the physico-chemical and biological properties of the pond water in a favorable range required for survival, growth and good fish quality. The main aim of the research is to determine the effects of the organic manures on haematological parameter of the fishes

MATERIALS AND METHODS

Source of Experimental Fish

The fingerlings were obtained from the State hatchery (Bagauda Fish Seed Multiplication Center), they were quarantined and acclimated for one week, fed *ad libitum* two times daily.

Fish Holding Facilities

Experiments were conducted in concrete tanks of 10m^2 with a depth of 1 meter. The tanks were scrubbed with powdered detergent and later flushed with caustic sodium hydroxide and then repeatedly flushed with fresh water.

A thin layer of soil was spread on the bottom of all tanks as described by Snow, *et al.*, (1984). Subsequently all the tanks were filled with water from the Dam at Bagauda Fish Seed Multiplication Center of Kano State ministry of Agriculture and Natural resources were the study was conducted in October, 2006 to March, 2007.

Experimental Diet

The composition of the experimental diet use as feed is presented in Table 1, proximate composition of diet and organic manure used was presented in Table 2

Table 1 Composition of Ingredients in Experimental Diet

Ingredients	% Composition
Cray fish	45
Ground corn	35
Cassava flour	15
Blood meal	3
Bone meal	0.5
Sodium chloride	0.5
Premix	0.25

Table 2 Proximate Composition of Supplementary Diet and Organic Manure

Parameters	Supplementary diet	PD	CD	RC
Crude protein	33.59	19.22	10.56	2.00
Crude fat	4.89	4.32	1.28	0.00
Crude fiber	9.02	7.54	5.62	21.00
Ash	13.88	13.98	15.46	12.00
Moisture	6.89	6.44	6.85	10.00
NFE	33.25	45.62	52.46	20.00

Experimental setting/ procedure

The experiment lasted for six months. Poultry droppings (PD), cow dung (CD) and rumen content (RC) were applied at three levels 30g, 60g & 120g/m^2/week, 25g, 50g & 100g/m^2/week and 22g, 44g & 88g/m^2/week treatment respectively, while in the control only conventional feeds was fed with 40% CP without manure application; fishes were fed with supplementary feed at 3% of fish biomass. Both manured and control groups were replicated three times. Each tank was stocked with fry (5 fish/m^2) of three fish species; *C. gariepinus*, *O. niloticus* and *C.carpio* (common carp) per pond, weight range of fishes at the time of stocking was 5.0 – 7.0g for *C. gariepinus*; 9 – 10g for *O. niloticus* and 8 – 9g for *C. capio*

Blood parameters Determination:

Haematological studies was carried out on the following parameters: Haemoglobin percentage, Red blood cell count, Partial and Total haemolysis on *O. niloticus, C. gariepinus* and *C. capio*. At the end of trial period blood samples were collected per treatment; in separate containers containing 0.2g Ethylene Diano Tetra Acetic acid (EDTA) as anticoagulant (Klontz 1972; Mawdesley-Thomas, 1972). Blood samples were collected by piercing in the ventro-lateral side of the caudal region with 1 ml disposable syringe. Blood smears were immediately prepared from each sample on microscope glass slides.

(a) The haemoglobin (Hb%) was determined by diluting 0.5 ml blood into 5 ml 0.4% ammonia solution in specimen bottles, using 1 cm cuvets blood samples compared with standard using the Grey-wedge photometer. The conversion was read in gram % on the Halden scale as described by Baron (1974).

(b) The red blood cell (RBC) enumeration was done using an improved Nueber ruling counting chamber, using Bayen's fluid (0.05 NaCl + 2.5g Na_2 SO_4 + 0.25 $HgCl_2$ made up of 100 ml with distilled water). The pipette was filled with blood up to the 0.5 mark, and was kept horizontal to prevent shifting the Bayen's solution and it was drawn to 101 mark. The content was mixed and the counting chamber covered with cover slip, the exposed part of haemacytometer was brought to contact with the tip of pipette, even distribution of cells was achieved by allowing sufficient fluid to run under the cover slip on the counting plat form, then examined under the microscope at 10× magnification, the corpuscles were counted in 1 mm^2 = 10,000 X Messager, *et.al.*, (1992) Where X = the number of RBC counted

(c) Erythrocyte fragility was determined through the micro method according to Messager, *et.al.*, (1992) using concentration of NaCl saline solution 10µl of heparinised blood were placed into 7 specimen bottles with round base NaCl varied from 1 to 7/1000, by steps of 1/1000, partial and total haemolysis of the cells per fish per treatment was confirmed.

RESULTS

The haematological parameters of *O. niloticus, C. gariepinus* and *C. capio* in polyculture under different manures treatments and control at three levels indicates that mean hemoglobin percentage increased from Rumen content treatment RC_2 with 7.00 to highest level of 12.63 in PD_1 and control, significant variation also exist between the fish species, as in Table 3. The mean value for *O. niloticus* was 7.45 for all the treatments, *C. gariepinus* records a mean value of 12.63 while *C. capio* records 7.39. The result is not statistically significant between levels on the same treatment but values obtained between fishes are significant.

C. capio having least percentage under treatment of Rumen Content followed by *O. niloticus* and *C. gariepinus* having the highest value at control and PD_1. In the higher manure levels the hemoglobin percentage of the three fish species under the treatments, the mean value for *O. niloticus* was 7.14 for all the treatments, *C. gariepinus* records a mean value of 10.01 while *C. capio* records 7.73 and mean per treatment with value of 7.05. In the CD_3 treatment which was double dose of CD_1 gives a hemoglobin percentage less value specifically on *C. gariepinus* and the response by the other fish species showed a decline in hemoglobin percentage Table 3, the result is not statistically significant between treatments but values obtained between fishes are significantly different.

The red blood cell count has its highest mean value in control treatment decreasing at a uniform rate from PD_1, PD_2 as well as CD_1, CD_2 and least value in RC_1 and RC_2 with lowest value of 0.40. The fish species also showed variance under different treatments with *O. niloticus* and *C. capio* having higher values at the same treatments of control and PD_1 are statistically significant. Red blood cell count for the fishes per treatment on Table 3 indicating that *O. niloticus* recorded mean value of 0.79 while *C. gariepinus* mean record of 0.98 and *C. capio* recorded a value of 0.66. In the higher dose CD_3, PD_3, and RC_3 red blood cell count for the fishes per treatment indicates that *O. niloticus* recorded a mean value of 0.81, while *C. gariepinus* mean record of 0.95 and *C. capio* recorded a value of 0.86, the mean per treatment was within the range of 0.80 and 0.99 in CD_3 and in control respectively table 3, and the red blood cell count were stabile on the CD_3, PD_3 and RC_3 treatments the value does not exceeds other lower concentrations

Mean partial haemolysis varied from 2.73 0/1000 to 4.83 0/1000 among the treatments highest, in control and lowest value at RC_2 treatment, variation equally exist between fish species showing a similar trend with other parameter, *C. gariepinus* having higher value followed by *O. niloticus* and *C capio,* Table 4 , the mean value for *O. niloticus* was 3.60 and for *C. gariepinus* 4.42 and *C. capio* recording a mean value of 3.06. The result of partial haemolysis in the higher levels has a mean per treatment within the range of 3.28 and 3.95 recorded in CD_3 and control respectively and the mean value of 3.21 Table 4.

Table 3 Red Blood Cell and Hemoglobin percentage of fish species under different manure treatments

	RBC (10^6 mm^{-3})			HB%		
Treatments	O.N	C.G	C.C	O.N	C.G	C.C
PD1	0.78b	1.12b	0.61b	7.47c	12.63a	7.22b
PD2	0.81b	1.03b	0.60b	7.22c	12.03b	7.21b
PD3	0.92a	0.92b	0.90a	7.23c	12.66a	7.24b
CD1	0.70c	1.03b	0.70b	7.73b	12.50a	7.21b
CD2	0.80b	1.03b	0.60b	7.73b	12.03b	7.03c
CD3	0.78c	0.92b	0.70b	7.74b	12.55a	7.25b
RC1	0.79b	0.87c	0.41c	7.10c	12.13b	7.03c
RC2	0.76b	0.88c	0.40c	7.07c	11.33c	7.00c
RC3	0.72c	0.78c	0.90a	7.15c	11.95b	7.50b
Control	0.87a	1.26a	0.83a	8.08a	12.63a	9.26a
Mean	0.79	0.98	0.66	7.45	12.63	7.39
LOS	0.01	0.90ns	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Means with common Alphabet in the same row are not significantly different (P < .005)
Oreochromis niloticus (O.N), *Clarias gariepinus*(C.G) and *Cyprinus capio* (C.C)
Poultry Droppings (PD), Cow Dung (CD) Rumen Content (RC)

Mean total haemolysis declined from 6.07 0/1000 in control to 4.03 0/1000 at CD_2 treatment. Total haemolysis result presented in Table 4. Total haemolysis result showed that the mean per treatment was within the range of 4.74 and 5.11 for CD_3 and PD_3 respectively, while the mean value recorded for *O. niloticus* was 4.86, *C. gariepinus* having mean value of 5.59 and *C. capio* recording a mean value of 4.36.

Table 4 Total and Partial Haemolysis of fish species under different manure treatments

	Total Haemolysis (%)			Partial Haemolysis (%)		
Treatments	O.N	C.G	C.C	O.N	C.G	C.C
PD1	5.08a	5.93a	4.43b	3.83a	4.72a	3.20b
PD2	5.07a	5.90a	4.47b	3.80a	4.69a	3.03c
PD3	5.20a	5.32b	4.82a	3.82a	4.20b	3.20b
CD1	4.93a	5.73b	4.38b	3.80a	4.83a	3.24b
CD2	4.63b	5.47b	4.03c	3.50b	4.80a	3.13bc
CD3	5.00a	5.20c	4.02c	3.20b	3.80c	3.00c
RC1	4.47c	5.53b	4.13b	3.57b	4.67a	2.53c
RC2	4.53b	5.63b	4.73a	3.53b	4.47b	2.73c
RC3	4.62b	5.46b	4.62a	3.23b	3.40b	3.20b
Control	5.08a	5.82a	4.04b	3.80a	4.62a	3.42a
Mean	4.86	5.59	4.36	3.6	4.42	3.06
LOS	< 0.0002	0.82ns	0.90ns	< 0.0002	0.90ns	0.82ns

Means with common Alphabet in the same row are not significantly different (P < 0.005)
Oreochromis niloticus (O.N), *Clarias gariepinus* (C.G) and *Cyprinus capio* (C.C)
Poultry Droppings (PD), Cow Dung (CD) Rumen Content (RC)

DISCUSSIONS

Various investigations during the past years have shown that the sensitivity of several haematological parameters to declining water quality and measurements of some biochemical parameters give good indication of tissue damage which result from pollution. In the present study most haematological parameters obtained in the treatments were within normal range for teleost blood parameters as reported by Blaxhall (1966).

Haematological indices (RBC counts, concentration of haemoglobin, partial and total haemolysis) have been reported to indicate secondary responses of an organism to pollutants (O'Neal and Weirich, 2001). The mean values of Hb% and Rbc, of *C.gariepinus* was higher than *O. niloticus* and *C. capio* even though there were no significant changes between the treatments. A slight increase and decrease in the concentration of haemoglobin in the blood, which is usually caused by the effect of nitrate on blood, as well as decreases in oxygen also indicates anaemia or confirms negative changes occurring in fish (Ali *et al*., 2008). Decrease in RBC count, partial and total haemolysis have also been reported by several workers after insecticide feeding (Mandal *et al*., 1986; Ali, 1989; Hamilton *et al.,* 1978). These Clearly indicate that the excessive water nutrients affect the haematological components of fish.

A number of works have reported nitrite uptake and toxicity mechanism in fish of freshwater environment. From the result of present study the nitrite concentration per manure treatment indicates higher value in control and poultry dropping followed by cow dung and rumen content showing a significant difference between treatments both on HB% and RBC with *C. gariepinus* having higher values than *O. niloticus* and *C. capio* this could be as a result of continuous consumption of the feeds in control and poultry manure, leaving little for decomposition and oxygen depletion. The result contradicts reports by Gill *et al*. (1991) that appearance of increase in RBC cells with increasing nitrate concentration leads to hypoxia since these are the component of blood for oxygen uptake and delivery, under hypoxia condition, fish attempt to adjust oxygen carrying capacity through increasing the surface area of erythrocyte (Soivio & Nikinma, 1981).

The progressive reduction in hemoglobin content in the present study may be attributed to the nitrite causing methaemoglobinemia as equally reported by several workers (Vedel *et al.,* 1998; Das, 2001;Huertas, *et al*., 2002; Martinez & Souza 2002; Jensen 2003).

The greater reduction in haemoglobin content in the fish species from *C. gariepinus* to *O. niloticus* and *C. capio* may be attributed mainly to the haemopoietic activity of kidney which is higher in *Claridae* family (Stormer *et.al*., 1996). Higher concentration of manure indicates none significance between treatment as well as between the three fishes. This may be attributed to the inherent abilities of the fishes to adjust their erythrocyte membranes to be more resistant to haemolysis in almost all the treatments. Haemolysis has been described in different fish species exposed to heavy metals (El-Domiaty, 1987, Annune *et.al*., 1994). Organic material at higher dose binds to serum albumin and Amino acids and passes readily to erythrocytes where it manifests damage (El-Domiaty, 1987). In this study higher concentrations of the manure PD_3, CD_3 and RC_3 treatments results in an increase in haemoglobin percentage in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis in all the fishes, as similar findings was reported by (Hodson, *et al*., 1978). Normal levels of hematological variables are important in utilization of hematological parameters in assessing the health status of fish or detecting if any disturbance has been introduced into the fish habitat as a result of pollution

CONCLUSIONS

Aquatic nutrients load undoubtedly has direct effects on fish health and survival. It can be concluded from the study that higher levels of manure 120,100 and 88 g/m^2/week affects haematological parameters. The study revealed that increase and decrease of various haematological of test fish samples explains the abilities of the fishes to withstand water conditions caused by the manure in the pond water. Therefore, there is need to fertilize pond with organic manure doses lower than 120,100 and 88 g/m^2/week for PD, CD and RC respectively for the benefit of diverse fish species and the condition of the ecosystem.

Conflicts of interest: The authors declare no conflict of interest.

Author's Contribution

This work was carried out by Yola, Idris Ado as a Ph.D Thesis and was supervised by Adikwu, I.A.

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