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Comparison of the haematology and injury profile of African catfish (*Clarias gariepinus*) transported in water treated with red palm oil or conventional anti-stress

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Abstract

This study was a comparative evaluation of the effects of adding red palm oil or a conventional anti-stress to water for transporting African catfish (*Clarias gariepinus*). One hundred and eighty apparently healthy matured Dutch strain of African catfish (also known as Dutch *Clarias*) of both sexes weighing 300 – 800 g were used for the study. They were randomly assigned into four treatments at a stocking rate of 2kg/L, as follows: Treatment 1 (T1) – no anti-stress agent added; Treatment 2 (T2) - Aqua-anti-stress® (conventional anti-stress) added at a dose of 1g/L; Treatment 3 (T3) – Red palm oil added at a dose of 1.5 ml/L; and Treatment 4 (T4) – Red palm oil added at a dose of 2.5 ml/L. These substances were added to water used to transport the fish for an 8-hour journey. Blood samples were collected from 15 fishes in each treatment group before transportation commenced, immediately after the 8-hour journey, and three days after the journey. Results showed that here were no significant ($p > 0.05$) variations between the treatment groups in their haematology before transportation commenced and immediately after the 8-hour journey. However, three days after the journey, the packed cell volume, haemoglobin concentration and erythrocyte counts of the T4 group was significantly ($p < 0.05$) higher than that of T3 group. The platelet counts of T4 group was significantly ($p < 0.05$) higher than those of all other groups. Monocyte counts of T1 group was significantly ($p < 0.05$) higher than those of all other groups. Percentage of fishes with physical injuries during transportation was lowest in the T4 group, followed by T2 group, then T3 and T1. It was concluded that addition of red palm oil at the levels used in this study minimized physical injury during transport of the African catfish, and that 1.5ml/L dose compared effectively with the conventional anti-stress on its effect on haematology.

Keywords: African catfish; Anti-stress; Transportation stress; Conventional anti-stress, Red Palm oil, Haematology.

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Introduction

Fish production has become an important economic activity in many countries, because fish and fishing products are important sources of protein and essential micronutrients for balanced nutrition and good health, and fish has been recognized as the cheapest source of animal protein in the diet of all, especially low income earners (Allison, 2001). Aquaculture is thus the fastest growing food production sector in the world, providing sufficient supplement of protein for people in many countries (Subasinghe and Bernoth, 2001).

The establishment of new fishery farms is on the increase throughout the world (Anetekhai, 2013). Thus, transporting fish by road is an inevitable phase in fish farm establishment and fish production. This commonly involves transporting fingerlings, brood-stocks or table size fish by road. Road transportation is the easiest and most convenient means of transportation to all possible places that fish farms need to be established. The process of road transportation is stressful, and there are ample reports in available literature on transportation stress in livestock and ways of managing it (Adenkola and Ayo, 2009; Adenkola and Onyeberechi, 2015; Bhatt *et al*., 2021). However, information on transportation stress and its management in fishes is scanty in available literature (Falaye *et al*., 2012).

The main aim of fish transportation is to deliver at the destination no matter the distance, healthy, active and alive fish without much physical injury. In the course of transportation of aquatic animals in general, the major concerns are usually the management of handling stress, mechanical shock, heat stress and the quality of the transport water (Coyle, *et al.* 2004; Crosby *et al.,* 2006). Several agents such as salt, palm oil, anesthetics, additives and other conventional anti-stress have been used intensively during fish transportation in order to alleviate transportation stress related problems (FishDoc, 2001; George et al., 2009; Christian and Jin-Liang, 2014; Martos-Sitcha *et al*., 2020; Chandararathna *et al.*, 2021). These agents are usually added to reduce stress and the level of physical injury or lower the extent of stress related mortality.

Haematological parameters have been widely used for detection of physio-pathological alteration in different condition of stress in livestock (Adenkola, *et.al.* 2010; Kandeepan, 2014). There is however a paucity of information on the haematological profile of transported fish. The aim of this study therefore was to comparatively evaluate the haematological and injury profile of African catfish transported with water in which was added red palm oil or a conventional anti-stress (Aqua anti-stress®).

Materials and Methods

Study Area: The study was carried out at University of Agriculture Makurdi (6^0 8' N, 7^0 10' E), located in Benue State, in the Southern Guinea Savanna area of Nigeria.

Experimental Fish and Management: One hundred and eighty (180) apparently healthy table-sized African catfish, also known as *Dutch Clarias* (*Clarias gariepinus*) of both sexes with weight ranging from 300 – 800 g were used for the study. They were purchased from a homestead fish farmer within Makurdi metropolis, Benue State, Nigeria. These fishes were harvested using 4 x 3 meter sieve net into two 500-litres plastic containers. The fish were further transported to the University of Agriculture, Makurdi fish farm where they were acclimatized for 7 days (Oriakpono *et al.*, 2012) inside 5 × 5 × 1 metre earthen pond before the experiment. The fishes were fed 2 times a day with commercial fish feed during this acclimatization. Before the day of the experiment, the fishes were fasted 24 hours and were also fasted throughout the experimental journey (NAERLS, 2001) to reduce the risk of regurgitation of food that may lodge in the gills of the fish and also decrease fecal contamination of the water used for the journey. The fishes were humanely handled all through the study.

Loading and Transportation of the Fishes: The 180 fishes were randomly assigned into twelve groups of fifteen fishes each, and each group was kept in a 25-litre capacity jerry can, giving a stocking rate of approximately 2kg/L of water (Falaye *et al.,* 2012). These twelve jerry cans represent four treatments in triplicate, as follows: Treatment 1 (Control) – No anti-stress agent was added to the transport water; Treatment 2 – A conventional anti-stress (Aqua anti-stress®) was added to the transport water for the fish at a dose rate of 1g/L of water, according to the manufacturer's instructions; Treatment 3 – Red palm oil was added at a dose of 1.5 ml/L (22.5 ml) to the transport water; Treatment 4 – Red palm oil was added at a dose rate of 2.5 ml/ L (37.5 ml) to the transport water. The dose of 1.5 ml/L and 2.5 ml/L were chosen for the study based on the outcome of earlier reported related study by Falaye *et al*. (2012). All other conditions in the three treatments groups remained the same as with the control. The fishes were then transported for the period of 8 hours covering a total distance 300 km. The vehicle was moving at the speed of 40 – 50 km/h. The fishes were also observed for injuries before and after the transportation, and these were recorded.

Blood sample collection: Blood samples were collected from 15 fishes selected randomly per group, before the journey, immediately after the journey, and three days after the journey. The blood samples were collected as described by Muntean and Marcus (2016). Two millilitre of the blood was collected from the fish and dispensed into a Bijou bottle containing EDTA as an anticoagulant for complete blood cell count using Vet Hematology Auto Analyzer (BC 2800 Vet Mindray®, U.K.). A separate syringe and needle was used for each fish.

Statistical analysis: All the data obtained were subjected to analysis of variance (ANOVA), and where significant variations existed, the means were separated using Duncan multiple range test. Data were expressed as mean ± standard error of mean (SEM). Values of $p < 0.05$ was considered significant

Results

The trend of changes in the hematological parameters of the transported fish is shown in Tables 1 – 3. The recorded values of hematological parameters before the experimental journey did not significantly vary ($p > 0.05$) between the treatment groups (Table 1). Also, immediately after the 8-hour journey, there were no significant variations in the haematological parameters between the four treatment groups (Table 2). However, three days after the journey, the packed cell volume (PCV), haemoglobin concentration, erythrocyte counts, platelet counts of the Treatment 4 group was significantly higher ($p < 0.05$) than that of Treatment 3 (Table 3). However, the platelet counts of Treatment 4 fishes was significantly ($p < 0.05$) higher than those of all other groups, while the monocyte counts of fishes in Treatments 2, 3 and 4 groups were significantly lower ($p < 0.05$) than that of the Treatment 1 Control group (Table 3).

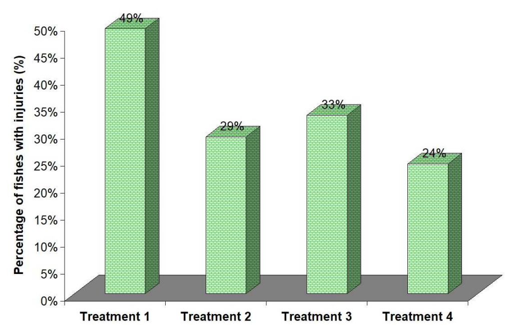

Figure 1: Percentage of fishes with injuries during the 8-hour transportation exercise in water in which were added Red palm oil or Aqua anti-stress®. [Treatment 1 (Control) – No anti-stress agent; Treatment 2 – Aqua anti-stress® (1g/L of water); Treatment 3 – Red palm oil (1.5ml/L); Treatment 4 – Red palm oil (2.5ml/ L)].

The level of injuries which affect the skin, barbell, tail, fins, head etc. were found to be highest in the Control (Treatment 1) group (49% injured), followed by the Treatment 3 group with a value of 33% injured, then Treatment 2 (29%) and Treatment 4 (24%) [Figure 1]. During the journey there was higher activity observed in the Control (Treatment 1) group than in all other treatment groups, with the Control (Treatment 1) fishes displaying vices such as cannibalism and restlessness, while there was a good level of calmness in the Treatment 4 group all through.

Table 1: Haematological parameters (mean ± SEM) of the African catfish used for the study before the experimental journey (baseline).

Parameter	Treatment 1 (Control)	Treatment 2 (Aqua anti-stress)	Treatment 3 (1.5ml/L Red Palm Oil)	Treatment 4 (2.5ml/L Red Palm Oil)
Packed cell volume (%)	44.65 ± 1.23	43.18 ± 1.76	42.93 ± 2.38	47.27 ± 1.61
Hemoglobin(g/l)	12.15 ± 0.29	11.60 ± 0.53	11.73 ± 0.43	11.78 ± 0.439
Erythrocyte count (10^{12}/L)	2.98 ± 0.071	2.85 ± 0.14	2.82 ± 0.11	2.86 ± 0.06
Mean cell volume (fl)	150.18 ± 2.74	151.80 ± 3.55	152.52 ± 5.06	165.55 ± 4.82
Mean cell hemoglobin (pg)	40.75 ± 0.26	40.60 ± 0.34	41.67 ± 0.53	41.28 ± 0.67
Mean cell hemoglobin concentration (g/L)	272.17 ± 5.28	267.67 ± 4.46	274.67 ± 7.83	260.33 ± 6.19
Red cell distribution with (%)	12.30 ± 0.20	12.97 ± 0.25	12.75 ± 0.28	13.15 ± 0.13
Platelets (10^9/L)	48.00 ± 4.44	49.83 ± 5.83	56.00 ± 4.08	54.67 ± 1.38
Mean platelets volume (fl)	5.18 ± 0.19	5.10 ± 0.13	5.52 ± 0.12	5.53 ± 0.12
Platelet distribution width (%)	19.27 ± 0.27	18.73 ± 0.22	19.55 ± 0.15	19.63 ± 0.16
Platocrit (%)	0.025 ± 0.004	0.024 ± 0.002	0.030 ± 0.003	0.030 ± 0.001
Leucocyte count (10^9/L)	193.02 ± 3.21	189.65 ± 6.20	187.23 ± 5.71	189.65 ± 3.11
Neutrophils (10^9/L)	37.76 ± 3.69	30.12 ± 4.03	31.97 ± 2.69	34.98 ± 2.18
Lymphocytes (10^9/L)	157.81 ± 2.98	158.93 ± 3.30	154.30 ± 6.30	153.11 ± 4.51
Monocytes (10^9/L)	0.00 ± 0.00	0.28 ± 0.28	0.34 ± 0.34	0.97 ± 0.43
Eosinophils (10^9/L)	0.33 ± 0.33	0.00 ± 0.00	0.62 ± 0.4	0.30 ± 0.30
Basophils (10^9/L)	0.00	0.00	0.00	0.00

Table 2: The haematological indices (mean ± SEM) of the fish transported in water treated with red palm oil or Aqua anti-stress, after the 8 hour experimental journey.

Parameter	Treatment 1 (Control)	Treatment 2 (Aqua anti-stress)	Treatment 3 (1.5ml/L Red Palm Oil)	Treatment 4 (2.5ml/L Red Palm Oil)
Packed cell volume (%)	40.93 ± 2.87	44.67 ± 2.07	42.30 ± 2.98	47.68 ± 2.24
Hemoglobin(g/l)	11.40 ± 0.78	11.93 ± 0.68	11.38 ± 1.09	12.42 ± 0.82
Erythrocyte count (10^{12}/L)	2.71 ± 0.20	2.85 ± 0.15	2.75 ± 0.23	2.78 ± 0.21
Mean corpuscular volume (fl)	151.75 ± 3.03	157.27 ± 2.67	155.25 ± 5.84	154.85 ± 5.26
Mean corpuscular hemoglobin (pg)	42.18 ± 0.49	41.78 ± 0.87	41.17 ± 0.57	41.95 ± 0.74
Mean corpuscular hemoglobin concentration (g/L)	261.83 ± 18.09	266.00 ± 4.95	267.33 ± 11.06	272.00 ± 7.12
Red cell distribution with (%)	12.35 ± 0.26	12.73 ± 0.25	12.53 ± 0.52	12.67 ± 0.26
Platelets (X10^9/L)	42.18 ± 5.28	52.17 ± 2.59	53.50 ± 8.36	47.67 ± 5.55
Mean platelets volume (fl)	5.47 ± 0.25	5.65 ± 0.18	5.72 ± 0.296	5.48 ± 0.10
Platelet distribution width (%)	19.12 ± 0.36	19.73 ± 0.22	19.67 ± 0.33	19.68 ± 0.21
Platocrit (%)	0.023 ± 0.003	0.029 ± 0.002	0.31 ± 0.006	0.026 ± 0.003
Leucocyte count (10^9/L)	185.45 ± 7.36	192.20 ± 6.61	187.25 ± 10.17	182.77 ± 10.01
Neutrophils (10^9/L)	31.76 ± 2.44	35.65 ± 3.47	35.48 ± 3.92	27.81 ± 2.17
Lymphocytes (10^9/L)	153.15 ± 7.150	154.98 ± 7.33	151.21 ± 8.39	154.12 ± 8.55
Monocytes (10^9/L)	0.28 ± 0.28	1.24 ± 0.39	0.30 ± 0.30	0.00 ± 0.00
Eosinophils (10^9/L)	0.27 ± 0.28	0.32 ± 0.32	0.26 ± 0.26	0.84 ± 0.38
Basophils (10^9/L)	0.00	0.00	0.00	0.00

Table 3: The haematological indices (mean ± SEM) of the fish transported in water treated with red palm oil or Aqua anti-stress, three days after the experimental journey.

Parameter	Treatment 1 (Control)	Treatment 2 (Aqua anti-stress)	Treatment 3 (1.5ml/L Red Palm Oil)	Treatment 4 (2.5ml/L Red Palm Oil)
Packed cell volume (%)	43.52 ± 1.19 [ab]	44.45 ± 2.34 [ab]	38.70 ± 2.62 [a]	50.28 ± 1.60 [b]
Hemoglobin (g/l)	11.60 ± 0.45 [ab]	11.73 ± 0.66 [ab]	10.22 ± 0.66 [a]	13.85 ± 0.57 [b]
Erythrocyte count ($\times 10^{12}$/L)	2.77 ± 0.09 [ab]	2.85 ± 0.13 [ab]	2.49 ± 0.17 [a]	3.38 ± 0.13 [b]
Mean corpuscular volume (fl)	155.30 ± 3.00	155.88 ± 3.81	155.67 ± 1.23	149.05 ± 1.62
Mean corpuscular hemoglobin (pg)	41.43 ± 0.36	40.97 ± 0.70	41.12 ± 0.46	40.87 ± 0.33
Mean corpuscular hemoglobin concentration (g/L)	267.67 ± 5.55	263.67 ± 7.61	264.17 ± 2.36	274.67 ± 4.86
Red cell distribution with (%)	13.27 ± 0.32	13.77 ± 0.48	13.85 ± 0.42	13.12 ± 0.37
Platelets ($\times 10^9$/L)	39.67 ± 2.33 [a]	43.67 ± 2.17 [a]	43.00 ± 3.54 [a]	74.00 ± 13.84 [b]
Mean platelets volume (fl)	5.73 ± 0.19	5.65 ± 0.11	5.88 ± 0.29	5.90 ± 0.21
Platelet distribution width (%)	20.27 ± 0.29	20.08 ± 0.25	20.00 ± 0.24	19.63 ± 0.22
Platocrit (%)	0.023 ± 0.002	0.024 ± 0.001	0.025 ± 0.003	0.043 ± 0.009
Leucocyte count (10^9/L)	197.23 ± 4.54	197.27 ± 0.73	180.52 ± 8.88	217.32 ± 3.52
Neutrophils (10^9/L)	17.71 ± 3.74	28.44 ± 3.73	18.67 ± 3.31	31.64 ± 6.08
Lymphocytes (10^9/L)	156.88 ± 16.71	167.82 ± 5.83	161.50 ± 8.90	183.55 ± 6.18
Monocytes (10^9/L)	6.84 ± 2.62 [a]	0.67 ± 0.42 [b]	1.23 ± 0.65 [b]	0.71 ± 0.71 [b]
Eosinophils (10^9/L)	0.00	0.34 ± 0.34	0.25 ± 0.25	1.49 ± 0.74
Basophils (10^9/L)	0.00	0.00	0.00	0.00

[a, b] Different superscript in a row indicates significant differences between the menas of the Treatment groups; $p < 0.05$

Discussion

The values of the haematological parameters obtained in this study before transportation was not significantly different among the various groups; this is thought to be as a result of the fact that the fishes in the different treatment groups were of similar age group and were maintained under the same conditions during acclimatization. However, the values recorded in this present study were slightly higher than that reported for *Dutch Clarias* by Okorie-Kanu and Unakalamba (2015), possibly due to difference in age, nutrition and environmental factors. However, immediately after transportation there was a drop in the values of packed cell volume, hemoglobin concentration and erythrocyte count in the Control (Treatment 1) group, while there was no noteworthy difference in those groups given conventional anti-stress and 1.5ml/L palm oil in water. Adenkola and Ayo, (2009) reported that road transportation of livestock induced free radical generation which causes increase erythrocyte osmotic fragility as a result of free radicals destroying membrane integrity of the erythrocyte; this might likely be the reasons for the decrease in packed cell volume observed in this study in Treatment group 1 which was not given any anti-stress. Though, free radical generation was not quantified in this study; free radical generation are normal occurrence associated with stress situation especially road transportation (Adenkola and Onyeberechi, 2015; Adenkola, et al., 2016).

Palm oil has been reported to contain a higher proportion of vitamin E which is rich in tocopherols and tocotrienols, and which serve as potent antioxidants that protect cellular membranes from free radical catalyzed lipid peroxidation (Burton and Ingold, 1989; Frankel, 1989; Budin *et al*., 2009; Sen *et al*., 2010; Ayisi and Zhao, 2014). It is thought that these antioxidants in red palm oil may have helped maintain the cellular membrane integrity of the erythrocytes.

The relative decrease in total leukocyte count as well as neutrophil count recorded in the Treatment 1 group immediately after the 8-hour transport is in contrast to the reports of Buckham-Sporer *et al.* (2008) who demonstrated leukocytosis in transported young bulls and that it is one of the biomarkers of transportation stress. The differences in leukocyte reaction between the fish in this study and beef cattle reported by Buckham-Sporer et al., (2008) could possibly be due to specie differences, while the higher leukocyte count recorded in the Treatment 2 groups could be due to the fact that the conventional anti-stress may contain inherent substances that facilitate the release of leukocyte from their pool in the body into the peripheral circulation. The variations found in the hematological indices across the experimental period in this study period are believed to be defensive mechanism against the environmental stress. This is in line with the position of Caruso *et al.* (2005) who stated that hematological parameters may act as physiological indicators to changes in external environment. The higher values recorded for the erythrocytic profile of the Treatment 4 group three days post-transportation may be attributed to haemoconcenration, possibly as a result of the higher dose of red palm oil added to their transportation water.

The lower level of activity and lowest percentage of fishes with injuries recorded in the Treatment 4 group suggests that red palm oil at the 2.5 ml/L reasonably mitigated stress more than the other dose (1.5 ml/L) and conventional anti-stress. There was no mortality in all the treatments, indicating that application of palm oil (at the two doses used for the study) as well as conventional anti-stress in water used for transporting catfish did not exercise a negative effect. This is in agreement with the work of Falaye *et al.* (2012) who reported that addition of palm oil to water during the transportation of *Clarias gariepinus* preserved the physical appearance and increased their level of calmness which was evident with smaller number of fish that sustained injuries in the groups transported with water in which red palm oil was added.

Conclusion

Addition of red palm oil to water during the transportation of African catfish at the levels used in this study (1.5ml/L and 2.5ml/L) minimized activity, induced calmness and reduced the percentage of fishes with physical injury during transportation. Treatment 3 with 1.5ml/L level of red palm oil addition to water compared favourably with the conventional anti-stress used in the study on its effect on haematology.

Conflict of Interest

The authors declare that there is no conflict of interest

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