GLOBAL JOURNAL OF PURE AND APPLIED SCIENCES VOL. 7, NO. 2, MARCH 2001:201-204
COPYRIGHT (C) BACHUDO SCIENCE CO. LTD. PRINTED IN NIGERIA. ISSN 1118-0579

COMPARISON OF HAEMATOLOGICAL INDICES IN JUVENILE CATFISH, *CHRYSICHTHYS NIGRODIGITATUS* (LACEPEDE), FED COMPLETE AND INCOMPLETE DIETS

S. B. EKANEM

(Received 3 April 2000; Revision accepted 15 December 2000)

ABSTRACT

Blood parameters of juvenile *Chrysichthys nigrodigitatus* were examined after the fish had been fed different feeds for 30 days. Clotting time, hematocrit, erythrocyte sedimentation rate, hemoglobin concentration, erythrocyte count and leukocyte count of fish from different incomplete feed treatments were compared to those of fish fed with control diet (complete feed). T-test found no significant difference ($P > 0.05$) in clotting time between groundnut cake treatment and the control diet but there were significant differences ($P < 0.05$) in other treatments. The non-significant difference in erythrocyte count of blood meal and palm kernel treatments indicates that their inclusion in feed could enhance erythrocyte production. Highly significant differences ($P < 0.01$) were found in leukocyte count between the control and the incomplete diets implying that incomplete diets reduce the production of leukocytes in fish blood. The zero sedimentation rate of erythrocytes in fish fed with fish meal and groundnut cake indicates that these feed components are outstanding ingredients for the composition of feed for the species.

KEY WORDS: **Haematological indices, *Chrysichthys nigrodigitatus*, diets.**

INTRODUCTION

The catfish, *Chrysichthys nigrodigitatus*, is widely used for intensive pond culture (Silvalingam 1976; Ezenwa 1982; Obiekezie and Enyenihi 1983). To get the best from this culture efficient feed must be produced for the species using local feed components; production of efficient feed entails knowing the effects of the different feed components on the species. Blood parameters affords a quick means for this determination.

Blaxhall and Daisley (1973) described various methods for determining fish blood parameters. They also pointed out the possibility of using these parameters to identify abnormalities in fish. Erythrocyte sedimentation rate (ESR) had been used to ascertain the response of blood to starvation and nutritional deficiencies in fish (Blaxhall 1972). Hilge (1978) examined the influence of different dietary protein and fat levels on some blood parameters in young mirror carp. With the use of blood and other parameters, Yurkowski (1986) was able to assess the suitability of two diets for Arctic charr (*Salvelinus alpinus*).

Abnormalities in fish due to deficiencies in diet can be detected by comparing the blood parameters of the affected fish with that of the control or with the normal blood profile if such is available. Etim *et al* (1999) made such profile available for *C. nigrodigitatus*. By comparing blood parameters of fish on different diets to those on complete (control) diet Yurkowski (1986) assessed the impact of the various diets. The present study compares the blood parameters of fish fed complete diet to those of fish fed incomplete diets with the aim of detecting the impact of each incomplete feed; each incomplete feed constituted a component of the complete feed.

MATERIALS AND METHODS

Fifteen healthy juvenile *C. nigrodigitatus*, stocked in each of five freshwater ponds (surface area 36m²), were fed with different feeds. One of the feeds, the control feed, was a complete diet compounded with 22.5% each of palm kernel cake, groundnut cake, fish meal and blood meal in addition to 3% vitamin/mineral premix and 3% vegetable oil; 4% maize meal served as a binder for all feeds. The other four feeds were incomplete diets, consisting predominantly (90%) of a single component of the complete diet. Common to all the feeds were 3% vitamin/mineral premix and 3% vegetable oil.

The juvenile catfish stocked for the experiment weighed between 15g and 136g and were of total length 11.9 - 24cm. The fish were fed once a day with 10% of their body weight for 30 days. After 30 days the fish were harvested and their blood parameters were examined. The pH and other physico-chemical parameters of the pond water, which could be

S. B. EKANEM: Department of Biological Sciences, University of Calabar, Calabar, Nigeria

controlled, were monitored and maintained at similar levels in all ponds.

Blood samples of five fish (total length 18 - 27.6cm and weighing between 66g and 149g), taken at random from each treatment, were used in the study and analysis. The parameters studied were clotting time, hematocrit, erythrocyte sedimentation rate (ESR), haemoglobin concentration, erythrocyte and leukocyte counts. The fish were anaesthetized in 1:15000 concentration of phenoxyethanol-2. Blood sample was collected from each fish after it had lost equilibrium. Collection was by cardiac puncture using 2ml sterile plastic syringe and 21 swg needle.

A little quantity of the blood from the syringe was introduced into a capillary tube, the time lapse between the introduction of the blood into the syringe and the time it started to clot was taken as the clotting time.

Blood was transferred from the syringe into a vial containing anticoagulant (potassium salt of ethylenediamine tetra-acetic acid, EDTA) to give a concentration of 5mg EDTA per ml of blood. Three capillary tubes were filled with well-mixed anticoagulant-treated blood from each fish and centrifuged for 5 minutes at 10500 rev/min. The percentage hematocrit was measured in each tube and the average obtained from the three tubes was recorded for each fish.

Three microhematocrit tubes were filled with blood from each fish group after sealing one end of the tube and allowed to stand for 10 minutes; this gave sufficient time for complete conversion of haemoglobin into cyanmethaemoglobin. Transmittance was read from a Corning colorimeter 253 at wave length of 540mm. This reading was then converted to haemoglobin concentration in mg/100ml by making reference to a graph constructed on available commercial cyanmethaemoglobin standards. Such readings for three blood samples were averaged for each fish group.

Erythrocytes and leukocytes were counted in a counting chamber on a microscope after the blood had been diluted with Dacie's fluid (Dacie and Lewis 1968) and stained with brilliant cresyl blue. For leukocyte count, dilution was 1:1500 and for erythrocyte count, a dilution of 1:2000 was used.

Mean and standard deviation for each blood parameter studied were calculated and students t-test was used to determine the deference between complete and the incomplete feed treatments.

RESULTS

Table 1 presents the blood parameters, their means and standard deviations obtained from fish in different treatments. Significant differences of the various parameters compared to those of the control group are also presented on the table. Data for erythrocyte sedimentation rate (ESR) of fish fed with palm kernel cake got lost but blood samples of fish fed respectively with groundnut cake and fish meal did not sediment at all.

DISCUSSION

Generally, longer clotting time signifies abnormalities (sickness) in fish, except the fish exposed to cobalt which had shorter clotting time (Kawatsu 1985). Significant differences in clotting time between the blood of the control group and those from each treatment has the following implications. Inclusion of fish meal in the feed could significantly reduce the clotting time whereas blood meal and palm kernel cake may increase blood clotting time of this species. The differences between the control and the various feed components (incomplete feed) suggests that the formulation of the control feed seems to be adequate in the proportion of its components. The lack of significant difference between fish fed with the control feed and those fed with groundnut cake seems to indicate normalcy in clotting time for this species cultured in pond and adequately fed. Clotting time obtained for these treatments and other treatments in this study are longer than normal given for this species by Etim *et al* (1999); this could be attributed to differences in the size of fish used in the two studies or their environments. The same could be said of other blood parameters in this study which were found to deviate from the normal profile. It also appears that blood parameters of cultured fish are different from those of wild fish which Etim *et al.* (1999) used in establishing the haematological profile for the species.

Non-significant difference between hematocrit value of the control group and blood-fed fish suggests that both treatments have similar effect on this blood parameter. Also fish meal and palm kernel cake seem to exert equal influence on this blood parameter. Groundnut cake is significantly higher in its contribution to the value of this parameter. Yurkowski (1986) linked low hematocrit value to deficiency of copper in the diet which hindered synthesis in the liver of Arctic charr. Thus, any value below that of the control group suggest inadequacy of the feed type used in this study.

The fact that erythrocyte sedimentation rate (ESR) is normally higher in diseased fishes than in healthy ones (Blaxhall and Daisley 1973) makes groundnut cake and fish meal treatments, each with zero sedimentation rate, stand out as important ingredient in composition of feed for this species. This single parameter favours the use of these components in feed formulated for this species. Many parameters in groundnut cake treatment were similar to the control, suggesting that the nutritive value of groundnut cake was closed to that of the control (complete) diet. Similarly, ESR of 1 ± 0.1 mm/hr obtained for blood meal treated fish was significantly lower than the value for the control group and is in favour of blood meal as feed component. The value for the control (2.6 ± 0.4 mm/hr) is similar to the normal value obtained from brown trout, *Salmo trauta*,

TABLE 1: Means and standard deviation of *Chrysichthys nigrodigitatus* blood parameters from different feed treatments

Treatment	Clotting Time (sec)	Hematocrit (%)	Erythrocyte Sedimentation Rate (ESR) mm/Hour	Haemoglobin Concentration g/100ml	Erythrocyte Count (x 10^6/ml)	Leukocyte Count (x 10^4/ml)
Control (Complete diet)	90 ± 7.9	20.4 ± 3.2	2.6 ± 0.4	5.1 ± 0.2	9.3 ± 1.1	64.55 ± 1.54
Palm kernel cake (Incomplete diet)	100.8 ± 1.9*	12.6 ± 0.9**		3.4 ± 0.3**	7.6 ± 3.8 ns	20.59 ± 0.41**
Groundnut cake (Incomplete diet)	85.6 ± 8.6 ns	24.4 ± 0.5**	0.0	5.9 ± 0.7 ns	5.9 ± 0.7**	36.77 ± 2.1**
Fish meal (Incomplete diet)	66.5 ± 2.6**	12.1 ± 0.1**	0.0	2.2 ± 0.2**	7.4 ± 0.7**	16.92 ± 1.1**
Blood meal (Incomplete diet)	106.8 ± 4.6**	17.0 ± 7.7 ns	1.0 ± 0.1**	3.8 ± 0.1**	8.6 ± 5.5 ns	11.72 ± 1.2**

* = Significant difference ($P < 0.05$)

** = Highly significant difference ($P < 0.01$)

n.s. = No significant difference between treatment and control ($P > 0.05$)

by Blaxhall and Daisley (1973); it is also close to the value obtained by Etim *et al* (1999) for this species in the wild.

The lack of significant difference between haemoglobin concentration in fish of the control group and fish fed with groundnut cake indicates that groundnut cake apparently exerts equal influence on this parameter as the control feed. The values and significant differences exhibited by other feed components point to their differences in nutritive value. Blaxhall and Daisley (1963) observed that the range in haemoglobin concentration of brown trout reflected the nutritional state of the fish. Hilge (1979) associated low dietary fat with low haemoglobin content in mirror carp. In this study haemoglobin concentration of 5.9 ± 0.7 g/100ml in fish fed with groundnut cake (33.3% fat) was not much different from that of the control (15% fat), nor was there difference between palm kernel treatment (7.5% fat). In this respect, the finding here contrasts with that of Hilge (1979). The contrast may be due to differences in species used.

Though erythrocyte count suffer some degree of error (Snieszko 1960), its value in this study shows that fish fed with palm kernel cake and blood meal respectively, were not significantly different from those fed with the control diet. This fact suggests that blood meal and palm kernel cake, as components of the feed, could contribute favourably to erythrocyte production in this species. Hilge (1979) found erythrocyte to increase with increasing fat content of young mirror carp. Such does not hold true in this study. Erythrocyte count of 5.9 ± 0.7 x $10^6 ml^{-1}$ obtained from groundnut cake treatment with the highest fat content of 33.3% in the feed was much lower than 9.3 ± 1.1 x $10^6 ml^{-1}$ and 8.6 ± 5.5 x $10^6 ml^{-1}$ obtained from the control diet and the blood meal diet respectively with 15% and 7.5% fat contents. The differences are probably due to the species used in this study and the one used by Hilge (1979).

Highly significant differences found in leukocyte counts of all incomplete diets compared to the control could mean that all incomplete diets significantly reduce

leucocyte production and hence reduction in resistance to attack.

The control groups in this study and in the study conducted by Arechon and Plumb (1983) on *Ictalurus punctatus* revealed remarkable similarities between the two species just like their external morphology does. Hematocrit value of 20.4 ± 3.2% in this study is within the range 22.5 ± 0.91% to 25.6 ± 1.6 obtained for *Ictalurus punctatus*. Similarly haemoglobin concentration of 5.1 ± 0.2 g/100ml got for *C. nigrodigitatus* in this study is not far from the range 5.35 ± 0.25 to 5.88 ± 0.26 g/100ml obtained for *I. punctatus* fingerlings. These two parameters suggest very close relationships between the two species which differ externally mainly in the number of anal fin rays and length of the dorsal fin.

CONCLUSION

The lack of statistical difference in clotting time between groundnut cake treatment and the control group indicate that the normal blood clotting time for this species under culture condition is 85.6 ± 8.6 to 90.0 ± 7.9 seconds. The highly significant difference in fish meal and blood meal treatments respectively imply shortening and lengthening of the clotting time respectively by their use as feed component; while zero erythrocyte sedimentation rate for groundnut cake and fish meal treatment make these feed components outstanding in feed composition for this species.

Non-significant difference in erythrocyte count of blood meal treatment and palm kernel cake treatment compared to the control diet indicate that their use as feed components for the species could enhance erythrocyte production in the fish. Also the lack of significant difference in haematocrit value and haemoglobin concentration in groundnut cake treatment is in favour of groundnut cake as a suitable component of feed for this species.

The differences in leukocyte counts of all treatment indicate that all incomplete diets significantly reduce leukocyte production leading to low resistance to attack.

Remarkable similarities were discovered in hematocrit value and haemoglobin content of *Chrysichthys nigrodigitatus* and *Ictalurus punctatus*.

ACKNOWLEDGEMENTS

I owe profound gratitude to the International Foundation for Science (IFS) who sponsored this study. The contribution made by Mr. P.E. Ikpe of the University of Calabar Medical Centre in blood analysis is gratefully acknowledged. Also acknowledged is the help rendered by my student Mr. A.J. Ottoh, in data collection. The Institute of Oceanography, University of Calabar, provided ponds and other facilities used for this study and therefore deserve special thanks.

REFERENCES

Areechon, N and Plumb, J.A. 1983. Pathogenesis of Edwardsiella ictaluri in channel catfish *Ictalurus punctatus*. J. World Maricul. Soc. 14:249 - 260.

Blaxhall, P.C., 1972. The haematological assessment of the health of freshwater fish. A review of selected literature. J. Fish Biol. 4: 593 - 604.

Blaxhall, P.C. and Daisley K.W., 1973. Routine haematological methods for use with fish blood. J. Fish Biol. 5: 771 - 782.

Dacie, J.V. and Lewis, K., 1968. Practical haematology 4th Ed. London: Churchill.

Etim, L., Ekanem, S.B. and Utin, A., 1999. Haematological profile of two species of catfish, *Chrysichthys nigrodigitatus* (Lacepede) and *Chrysichthys furcatus* (Gunther) from the Great Kwa River, Nigeria: Global Journal of Pure and Applied Sciences 5(1): 1 - 4.

Ezenwa, B.I.O., 1982. Production of catfish *Chrysichthys nigrodigitatus* in brackish water ponds in Nigeria using groundnut cake as supplemental feed. Aquaculture, 27: 197 - 203.

Hilge, V., 1978. The influence of different dietary protein and fat levels on some blood parameters in young mirror carp (*Cyprinus carpio* L.) In: Proc. World Symp. on Finfish Nutrition and Fish feed Tech. II: 173 - 179.

Kawatsu, H., 1969. Studies in the anaemia of fish 3. An example of macrocytic anaemia. Bull. Freshwat. Fish Res. Lab., Tokyo 19: 161 - 167.

Obiekezie, A.I. and Enyenihi, U.K., 1988. Henneguya chrysichthys sp. nov. (Protozoa Myxozoa) from the gills of estuarine catfish *Chrysichthys nigrodigitatus* (Lacepede) (Pisces: Bagridae) in Nigeria. J. Af. Zool. 102: 33 - 42.

Silvalingam, S., 1979. The biology of cultivable brackish water and marine finfish in Africa. In: Symp. on Aquaculture in Africa. CIFA Techn. Paper No. CIFA/T4 (Suppl.1): 283 - 291.

Yurkowski, M., 1986. Suitability of two rainbow trout (*Salmo gairdneri*) reference diets for Arctic charr (*Salvelinus alpinus*). Can. Tech. Rep. Fish Aquat. Sci. 1464: IV + 10p.