Direct Research Journals
Vol.5 (6), pp. 257-262, June 2017
ISSN 2354-4147
International Standard Journal Number (ISJN) e-ISJN: A4372-2604
Article Number: DRJA021863974

Direct Research Journal of Agriculture and Food Science
http://directresearchpublisher.org/aboutjournal/drjafs

Research Paper

Haematological indices and some biochemical enzymes of *Clarias gariepinus* (Burchell 1822) fed diets containing *Ageratum conyzoides* leaf meal

Ebochuo V.C.[1]* and Oparaejiaku, J.[2]

[1]Department of Fisheries and Marine Technology, Imo State Polytechnic, Umuagwo, Ohaji, Nigeria.
[2]Department of Agriculture Management and Economics Technology, Imo State Polytechnic Umuagwo,Ohaji, Nigeria.
*Corresponding author E-mail: victorebochuo@gmail.com.

Received 7 May 2017; Accepted 16 June, 2017

This study was conducted to determine the effect of *Ageratum conyzoides* based diets on the haematology and some biochemical enzymes of *Clarias gariepinus* juveniles. Ninety fish were assigned to the three experimental diets in which *A. conyzoides* leaf meal replaced wheat bran at 0%, 50%, and 100% respectively at 30 fish per treatment which was further sub-divided into 3 replicates of ten fish each in a completely randomized design. Range of values for PCV are 20.07%-20.67%, and were not significantly different (P>0.05). A significant WBC value (4.70 ±0.20x10^{3}mm^3) was produced by 100% *A. conyzoides* leaf meal diet. RBC was significant (P> 0.05) at 50% and 100% *A.conyzoides* leaf meal diet over control. HB was similar in all treatments (Range 6.77 ±0.50 – 6.90± 0.30). Lymph level in *A. conyzoides* based diets were significantly (P<0.05) different from the control fed fish. MCHC, MCH and MCV values were lower than the normal healthy *Clarias gariepinus* juveniles. ALT, AST values were quite low and do not suggest cellular damage. ALP is however higher in both control and the diets. Results indicate that with proper treatment of *A. conyzoides* leaf meal, it could be incorporated in fish feed at even 100% with good results.

Key words: Clarias gariepinus, *Ageratum conyzoides leaf* meal, response, haematology and biochemical enzymes.

INTRODUCTION

Fish culture has attained the reputation of the fastest growing sector of Animal production posting an increase of 10% annually (Ozovehe, 2013). This kind of increase must be matched with commensurate fish feed production. Feed ingredients have become very expensive. The qualities of these ingredients are not assured and there is no guarantee as to their all season availability. In order to attain profitability, sustainable and ecologically friendly production, research interests have been directed towards evaluation and use of unorthodox ingredients in replacement experiments. While fish nutrition emphasizes the formulation of diets that stimulate optimum growth in minimum time, care is taken to minimize any form of compromise to the physiological health status of fish. This position has limited the use of those ingredients which are known to possess anti nutrients because they are lethal to fish and other monogastric animals. The third word is faced with the challenge of using unconventional feedstuff to replace Cereal based ingredients because they are cheaper and are not in any form of competition by man and industry. Forage plants fall into this category and among these is the plant, *"Ageratum conyzoides* L." It is known to have Ethnomedical applications and belongs to the family

Astarecea. It is an annual herbaceous plant with a long history of traditional medicinal uses in several countries of the world.

Haematological components are valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1988). Most works on *Ageratum conyzoides* are centered on evaluating toxicity in rats for biochemical and pharmacological reasons (Igboasoiyi *et al*., 2007 and Agunbiade *et al*., 2012). The numerous uses of *A conyzoides* have been enumerated (Dalziel, 1937; Menut *et al*., 1993; Kamboj and Sulaja, 2008). As an herbaceous forage plant, its' impact on Haematology and Serum Biochemistry of recipient organism deserves evaluation and mention. Toxicity studies on Ethanol extract of *A. conyzoides* in Albino Rats does not appear to have significant toxicity (Sumalatha, 2012). Biochemical, haematological and histopathological studies of extracts of *Ageratum conyzoides* in Sprague Dawly rats found significant increases in WBC counts, mean platelet volume and plaletet distribution width with significant reduction in ALP, AST and ALT activities (Adebayo *et al*., 2010). Haematopoietic properties of ethanolic leaf extracts of *A. conyzoides* in albino Rats show that they could possible remedy Anaemia (Ita *et al*., 2007).

The literature reviewed revealed that no studies have been carried out on the haematological and biochemical indices of *Clarias garieepinus* fed *Ageratum conyzoides* leaf-meal based diet. The aim of this work is to evaluate the Haematological indices and some biochemical enzymes of *Clarias gariepinus* fed diets containing varying levels of *Ageratum conyzoides*.

METHODS

Sampling collection and processing

The test ingredient (*Ageratum conyzoides* leaf meal) used for this study was collected from Imo State Polytechnic Umuagwo, Ohaji environment, Southeastern Nigeria and dried under room temperature for two weeks without exposure to sun. This was pulverized with a petrol engine hammer mill to fine powder and packaged in an air-tight polythene bag.

Feed formulation

Ageratum conyzoides leaf meal was mixed thoroughly with other feedstuffs (Table 1) to produce three isoproteic (CP 40%) diets such that Wheat Bran was gradually replaced by *Ageratum conyzoides* leaf meal at 0%, 50%, and 100% respectively. The control diets (0%) contained no *Ageratum conyzoides* leaf meal. Diets so produced were passed through a pelleting machine with 2 mm die to produce fish pellets, sundried for 4 days until crispy to prevent growth of mould on the feeds. The pelleted diets were packaged in water-proof nylon bags and were labeled accordingly prior to storage at room temperature. Samples of the diets produced were subjected to proximate analysis using standard methods as outlined by AOAC, (2000) and presented in (Table 2).

Experimental fish and design

A total of 90 African catfish juveniles with mean body weight 6.19 ± 0.1 g were purchased from a commercial hatchery at Owerri, south Eastern, Nigeria. They were acclimated for two weeks and fed the commercial diet in a plastic bowl. Subsequently, experimental fish were randomly assigned to the three diets at 30 fish per treatment in a completely randomized design. Each treatment was replicated thrice making a total of ten fish per replicate. Fish were fed the trial diets for a period of 8 weeks at 3% biomass shared between 8-9 am and 5–6 pm daily. The fish were reared in plastic containers measuring 80 L.

Blood and serum collection, haematological and biochemical analysis

Blood samples were collected at the 8th week of the experiment respectively. Two fish were taken from each of the replicates and anaesthetized following the methods of Osuigwe *et al.* (2005). Blood samples were collected following the procedure of Klontz and Smith, (1968) and Wedemeyer and Yasutake, (1977). Blood samples were collected in triplicate into sample bottles containing Ethylene Diamine Tetra Acetic acid (EDTA) as anticoagulant to give a concentration of 5mg/ml of blood sampled.

The blood sample was rocked gently in the bottle to allow thorough mixing of its contents. The blood samples collected were taken to the Haematology Laboratory of Federal Medical Centre, Owerri, for haematological assessment such as Haemoglobin (Hb), Packed Cell volume (PCV), Red Blood Cell (RBC), White Blood Cell (WBC), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC).

Serum collection and biochemical analysis were as above and according to Wedemeyer and Yasutake, (1977). The methods (Ogbu and Okechukwu, 2001) were also adopted.

Alkaline phosphatase (ALP) activity was performed using the modified method of Wright *et al*. (1972), Aspartate amino tranferase (AST) and Alanine amino transferase (ALT) activities were carried out according to the methods described by Reitman and Frankel, (1957).

Table 1. Composition of experimental diets fed to African catfish juveniles.

Ingredients	Control (0%)	Treatment 1 (50%)	Treatment 2 (100%)
A. conyzoides leaf meal	0	5.85	11.69
Wheat bran	11.69	5.85	0.00
Soya bean meal	34.84	34.84	34.84
Maize	11.69	11.69	11.69
Fish meal	34.84	34.84	34.84
Bone meal	02.00	02.00	02.00
Oil	03.00	03.00	03.00
Salt	00.25	00.25	00.25
Premix	00.25	00.25	00.25
Methionine	00.25	00.25	00.25
Lysine	00.25	00.25	00.25
	100.00kg	100.00kg	100.00kg

Table 2. Proximate composition of the experimental diets.

Parameters	Dietary inclusions		
	T1	T2	T3
Moisture content	9.50	10.5	9.00
Ash	5.10b	6.10a	5.00b
Crude fat	7.60a	7.40a	4.80b
Crude fibre	7.33a	08.00a	9.00b
Crude protein	39.56a	40.02a	39.67a
Nitrogen free extract (soluble carbohydrate)	30.91	28.98	32.53

Statistical analysis

All data collected were subjected to analysis of variance (ANOVA). Comparisons among diets means were carried out by Duncan Multiple Range Test (Duncan, 1955) at a significant level of 0.05. All computation was performed using statistical package SPSS 21.0 (SPSS Inc., Chicago, IL USA).

RESULTS

Table 3 summaries the results of the haematological indices of fish fed *Ageratum conyzoides* leaf meal based diet during the experiment.

Packed cell volume

The packed cell volume (PCV) showed that the fish fed 0%, 5% and 100% *A. conyzoides* leaf meal had values of 20.07%, 20.33% and 20.67%. These values are not statistically significant (P>0.05) from each other.

White blood cell

WBC showed that the fishes fed 0% and 50% *A. conyzoides* leaf meal had similar (P>0.05) values. The fish feed 100% *A. conyzoides* leaf meal was the highest showing a significant (P>0.05) difference statistically.

Red blood cell

The RBC showed an increase from the fish fed 0% to the fish fed 50% (P>0.05) and also increased up to the fished fed 100%. The fish fed 100% was increased significantly higher (P>0.05) than the fish fed 0%.

Haemoglobin

The HB levels in all diets from 0% to 100% *A. conyzoides* leaf meal were similar ranging from 6.77-6.90%. These values are not statistically significant (P>0.05).

Neutrophils

The Neutrophils level was significantly highest in fish fed 50% *A. conyzoides* leaf meal diet. All diets produced significantly (P>0.05) different values with the least being scored by control diet.

Mean cell haemoglobin concentration

Similar values (P>0.05) were also obtained with 0%, 50% and 100% *A. conyzoides* based diets. The values ranged from 32.83g/dl – 33.93g/dl.

Mean cell haemoglobin

The highest significant P>0.05 for MCH (pg) was obtained with diet with 0% inclusion of *A. conyzoides* leaf meal while the lowest was with the diet containing 100% inclusion of *A. conyzoides.*

Mean cell volume

The trend was similar with MCV (fl) which decreased from 0% to 100% inclusion levels of *A. conyzoides*. The result was statistically different.

Alkaline phosphatase (ALP)

The Alkaline Phosphatase (ALP) results showed that all values produced by 0%, 50% and 100% of *A conyzoides* diets ranged from $53.67UL^{-1}$ – $59.\ 33UL^{-1}$. All values varied significantly (P>0.05).

Aspartate aminotransferase (AST)

The Aspartate Amino Transferase (AST) result obtained ranged from $7.00UL^{-1}$ $10UL^{-1}$ and 0%, 50% and 100% *A. conyzoides* produced statistically (P>0.05) different results.

Alamine aminotransferase (ALT)

The result of the Alamine Amino Tranferase (ALT) showed that 50% *A. conyzoides* based diet was significantly higher that 0%, 100% *A. conyzoides* diet.

DISCUSSION

Heamatological Indices and Serum Biochemical parameters are often used to monitor the health status and stress indicators in fish. The present study was undertaken to assess the effect of diets containing 0%, 50% and 100% *Ageratum conyzoides* as a replacement for Wheat meal on *Clarias gariepinus* juveniles (Table 3).

Results of this work show that packed cell volume (PVC) of fish receiving the experimental diets produced similar results which were not statistically different. The range of PCV values from 0% - 100% diet were 20.07±1.00%-20.67±2.10%.

These are slight but statistically insignificant increases in PCV. PCV is the Erythrocytic Volume Fraction (EVF) which is the volume percentage of Red blood cells (RBCs) in the blood. It is a reference of the blood ability to deliver Oxygen.

A. conyzoides in diet could be said to improve PCV slightly and enhance fish health status since there are slight though insignificant increases over the 0% diet. Moreover the PCV recorded in this work is within the acceptable range of 18.9± 14% (Mohamed and Agad,2005), 30.08 ± 7.78% (juveniles from Benue River Nigeria (Bolade *et al*., 2014) and 31.22± 0.96%, young adults (Sontakke *et al*., 2014) (South Eastern Nigeria).

The white blood cells (WBC) encountered proved that fish fed 0% and 50% *A. conyzoides* based diets had similar (P>0.05) counts and differed statistically (P<0.05) from 100% *A. conyzoides* based diet. White blood cells (WBCs) are cells of the immune system that are involved in protecting the body against infectious diseases. The number of WBC in often an indicator of diseases meaning that higher values may prove the presence of disease organisms or toxins. The low values obtained in this study may be further proof of the antibiotic properties of *A. conyzoides*. Inclusion of *A. conyzoides* may therefore prove useful if it is incorporated into the production of medicated feeds for fish prophylaxis and therapuetics. The range of values for healthy *C. gariepinus* juveniles in other works were 18.25 ± 0.69 x 10^3/ml (Alikwe *et al.,* 2014), 25.26 ± 0.14 x 10^3/ml Omitoyim (2006) and 15.50± 1.15 x 10^3/ml (Bolade *et al*., 2014).

The Red blood cell count of fish increased as the inclusion level increase from 0% to 100% inclusion levels of *A. conyzoides* leaf meal A. statistical significance (P< 0.05) was established between 0% and 100% inclusion levels (Table 3). Erythrocytes are the most common type of blood cells in vertebrates. It is the principal means of delivering oxygen to body tissues via blood flow. Anemia may be imminent due to inability of RBC to carry enough Oxygen due to low count and Iron deficiency Anemia occurs when dietary intake or absorption of iron is insufficient and heamoglobin cannot be formed. High levels RBC synthesized here may be due to the high iron content in the plant *A. conyzoides* Agunmbiade *et al*. (2012) reported iron (Fe) content of 161mg/kg of *A.* conyzoides leaf meal. Again the levels present in the diets studied are higher than 2.17±3x10^6 /mm^3 (Mohamed and Agad, 2005), 1.72 ± 10.34 x 10^6/mm^3 (Bolade *et al.,* 2014), 2.4 ± 0.03 x 10^6/ul (Sontakke *et al*., 2014) for healthy juvenile *C. gariepinus* here show that *A. conyzoides* in diets can enhance RBC formation and hence overall blood production in *C. gariepinus*. Haematopoietic properties of ethanolic leaf extracts of *A. conyzoides* in albino Rats show that they could possibly remedy Anaemia (Ita *et al*., 2007).

The Haemoglobin values obtained with diets containing 0%, 50% and 100% inclusion levels of *A. conyzoides* were similar (P> 0.05). This equated the values obtain by Mohammed and Agad, (2005) but were lower than those of (Bolade *et al*., 2014; Omitoyim, 2006). The levels found here were also close to the values obtained with *Moringa Oleifera* based diets for *C. gariepinus* (Ozovehe, 2013). These values are therefore adjudged to be within the accepted range for *C. gariepinus* and can attest to good transport of oxygen and removal of carbon IV oxide from fish tissues.

The Mean Cell Corpuscular Haemoglobin concentration (MCHC) in all diets and at all inclusion levels (0%, 50% and 100%) of *A. conyzoides* were similar (P> 0.05). This is a measure of concentration of Haemoglobin in a given volume of packed red blood cells. It is usually regarded as part of the standard blood count. It towed the pattern of similarity reflected by Haemoglobin in level of significance. The result falls within the recommended range of 32 to 36g/dl and the findings of Bolade *et al*.

Table 3.Haematological parameters of C*larias gariepinus* juveniles fed different levels of *Ageratum conyzoides* leaf meal based diet.

Blood parameters	Control, 0% Ac	Diet 1, 50% Ac	Diet 2, 100% Ac
PCV%	20.67±1.06^{a}	20.33±1.20^{9}	20.67±2.10^{9}
WBC ($10^{3}mm^{3}$)	02.26±0.40^{a}	02.37+0.20^{a}	04.70+0.40b
RBC ($10^{6}mm^{-3}$	03.53±0.04^{ab}	04.33±0.20^{bc}	04.80±0.20^{c}
Hb (g/100ml)	06.77±0.50^{a}	06.90±0.30^{a}	06.87±0.40^{a}
Lymph %	89.33±10.00^{a}	77.66±5.10^{b}	85.67±3.00^{c}
Nuetrophils %	10.67±1.00^{a}	22.33±4.10^{b}	14.00±1.50^{c}
MCHC (g)	32.83±2.01^{a}	33.93±2.00^{a}	33.40±2.00^{a}
MCH(pg)	19.27±2.01^{a}	16.03±2.30^{b}	14.40±1.00^{b}
MCV (fl)	59.13±1.50^{a}	47.33±2.30^{b}	43.57±3.00^{c}

Table 4. Serum Enzyme Indices of C*larias gariepinus* juveniles fed different levels of *Ageratum conyzoides* based diet.

Parameters	Control 0%Ac	Diet 1 50%Ac	Diet 2 100%Ac
ALP(UL^{-1})	58.00±2.00^{x}	53.67±1.00^{y}	59.33±2.00^{x}
AST(UL^{-1})	08.00±0.20^{a}	07.00±0.10^{b}	10.00±020^{c}
ALT(UL^{-1})	06.66±0.50^{a}	08.33±1.00^{b}	06.00±0.40^{a}

(2014) (32.61± 10.42g/dl), (Sontakke *et al*., 2014) (35.0 ± 1.34g/dl), Omitoyin (2006) (33.10± 0.06g/dl) for healthy *Clarias gariepinus* juveniles. This again fuels strong support for inclusion of *A. Conyoides* in diets of *C. gariepuins.*

Mean Corpuscular Haemoglobin (MCH) values observed in this study reduced significantly (p<0.05) from 0% to 50% and to 100% inclusion levels of *A. conyzoides* in Diets of *C. gariepinus*. The values were much lower than those recorded elsewhere (Omitoyin, 2006; Mohammed *et al*., 2014). MCH is diminished in Hypochromic Anemia. If this situation should arise with *Ageration Conyzoides* based diets in *Clarias ganepinus,* it is an indication of Iron deficiency. Iron deficiency can only arise as a result of presence of Tannin and other untreated anti nutrients which may interfere with or bind proteins and other substances perhaps making iron unavailable or may make food unpalatable and hence reduce feeding (Harold, 2004) at high inclusion levels. This emphasizes the need to pre-treat raw *A. conyzoides* prior to inclusion in fish diet.

There is significant (P<0.05) uniform reduction in Mean Corpuscular Volume as amount of *A. conyzoides* increased from 0% to 100% which indicates an inverse relationship. Values obtained in both control and *Ageratum conyzoides* based diets are lower than the values for healthy *Clarias gariepinus*. It may still be associated with high levels of anti-nutrients in *Ageratum conyzoides* based diets. It is expected that improved processing methods can reduce anti-nutrient levels in formulated feed and improve the two of increased food intake and chelating of useful components of diet making them unavailable to fish. Osuigwe *et al*. (2005), had similar experience with anti- nutrients when jack beans was fed to *C.gariepinus*.

Alkaline phosphatase (ALP) values for diets of *Clarias gariepinus* diets containing *A. Conyzoides* at 0%, 50% and 100% inclusion levels were similar at 0% and 100% levels. Diet containing 50% *A. conyzoides* was significantly lower than other diets. Even the least result in this study is higher than that reported for normal and healthy *Clarias gariepinus* juveniles (5.29 ± 051Iµ) (Sarwar *et al*., 2012).

High ALP activity may be attributed to hepatic cellular damage leading to leakage into the circulation. Ozovehe, (2013) experienced the same with *C. ganiepinus* fed *Moringa oleifera* diets at over 30% and above inclusion levels.

Though there were significant differences in Alanine amino acid transferase (ALT) and Aspartate Amino acid transferase (ASP) in 0%, 50% and 100% *A. conyzoides* based diets, the values were quite low compared to what was reported for Healthy *Clarias* (ASP, 54.77 Iµ) Juveniles (Sarwar et al., 2012). This Observation shows that *Ageratum conyzoides* could not have induced cellular damage indeed but ALP activity may have been involved in its major function of phosphorylation (Table 4).

Conclusion

Results obtained in this study indicate that *A.conyzoides* could be incorporated into the diet of *C. gariepinus* at up to 50% level and even 100% if it is properly processed to eliminate the effect of anti-nutrients.

REFERENCES

Adebayo AH, Zeng GZ, Fan JH, JI CH, He WJ, Xu JJ, Zhang YM, Akindahunsi AA, Kela R, Tan NH (2010). Biochemical, haematological and histopathologicalstudies of extract of *Ageratum conyzoides* L. in sprague Dawley rat. J. Med. Plants Res. 4(21):2264-2272.

Alikwe PCN, Yeigba, J., Akinnus B.i, Oyenike F. A. and Ohimain E. I.(2014).Performance and Carcass Characteristics of Giant African Land Snails Fed *Alchornea Cordifolia* Leaf Meal in Replacement for Soybean Meal International Journal of Research In Agriculture and Food Sciences . Vol. 1, No.6

AOAC(2000).Official Methods of Analysis. (Ed. Herrick K), 17th Edition. Washington, DC, USA.

Dalziel JM (1937). The useful plants of West Africa. London: Crown Agents;

Duncan DB (1955). Multiple range and multiple F tests. Biometrics 11: 1-42.

ITA SO, Etim OE, Ben EE, Ekpo OF (2007). Haematopoietic Properties of Ethanolic Leaf Extract of *Ageratum conyzoides* (Goat Weed) in Albino rats.Nigerian Journal of Physiological Sciences 22 (1-2): 83-87 ©Physiological Society of Nigeria,

Kamboj A, Saluja AK (2008). *Ageratum conyzoides* L.: A review on its phytochemical and pharmacological profile. Int. J. Green Pharm. 2:59-68

Klontz GW and Smith LS (1968). Method of using fish as biological research subjects. In: methods of animal experimentation volume iii (ed. W.I. Grey), 323- 383. Academic press, New York.

Menut C, Lamaty G, Amvam PH (1993). Aromatic plants of tropical central Africa part X: Chemical composition of essential oil of *Ageratum houstonianum*. Flav. Frag. J. 8:1.

Mohamed HA and Agab H (2005). Haematological and biochemical indices of Clarias garipeinus collected from River Nile, Sudan. A Publication of the University of Science and Tech. Sudan.

Ogbu SI and Okechukwu FI (2001). The effects of storage temperature prior to separation on plasma and serum potassium. J. Med. Lab. Sci. 10: 1-4..

Omitoyin BO (2006). Haematological changes in the blood of Clarias gariepinus (Burchell, 1822). Juvenile fed poultry litter. Livestock Research for Rural Development. 18(11):1-6.

Osuigwe DI, Obiekezie AI, Onuoha GC (2005). Some haematological changes in hybrid catfish (*Heterobranchus longofilis* x *Clarias gariepinus*) fed different dietary levels of raw and boiled jackbean (*Canavalia ensiformis*) seed meal. *Afr. J.Biotechnol*. 4(9): 1017-1021.

Oyawoye EO, Ogunkunle M (1998). Physiological and biochemical effects of raw jack beans on broilers. Proceedings of annual Conference of Nigerian Society of Animal production 23: 141-142.

Ozovehe BN (2013). Growth Performance, Haematological Indices and Some Biochemical Enzymes of Juveniles *Clarias gariepinus* (Burchell 1822) Fed Varying Levels of *Moringa oleifera* Leaf Meal Diet. J. Aquac. Res. Development 4:166

Reitman S, Frankel S (1957). A Colorimetric method for the determination of glutamic–ozaloacetic and glutamic–pyruvic transaminases. Am. J. Clin. Pathol. 28: 56-63.

Sontakke U, KaleV, Bose B and Kumar M (2014). Non-Conventional Feeds and Agro Industrial By Products: Their Scope and Future Demand for Livestock Production. Dairy Cattle Nutrition Division National Dairy Research Institute. Technical Article, 3427, 9.

Sumalatha k (2012). Toxicity studies on Ethanol Extract oF *Ageratum conyzoides L.* in Albino Wistar Rats Journal of pharmaceutical biology, 2(1), 7-11.

Sarwar GG, Wu XC and Cockell KA (2012). Impact of antinutritional factors in food proteins on the digestibility of protein and the bioavailability of amino acids and on protein quality. Br. J. Nutr.108 Suppl. 2:S315-32.

Wedemeyer GA and Yasutake WT (1977). Clinical methods for the assessment of the effects of environmental stress on fish health. United States Fish and Wildlife Service, Federal Government Series, Technical papers pp: 89.

Wright PJ, Leathwood PD, Plummer DT (1972). Enzyme in rat urine: Alkaline phosphatase. Enzymologia 42: 317-327.