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Impact of parasites on the haematological and biochemical parameters of selected bagrid species from lower river Benue Nigeria

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Abstract
Impact of parasites on the haematological and biochemical parameters of selected bagrid species from Lower River Benue Nigeria was investigated. Out of the 240 fish samples comprising of 120 each of *Bagrus bayad* and *Bagrus docmak* used, 145 fish samples comprising of 46.21% samples of *B. bayad* and 53.79% samples of *B. ducmack* were not infested while 95fish samples comprising of 54.74% samples of *B. bayad* and 45.26% samples of *B. docmak* were infested with 389 different parasites belonging to one species of protozoan (*Tricodina acuta*) found on the gills and skin of their fish samples, two species of Cestode (Diphilobothrium latum and *Hymenolepis nana*), two species of Nematode (*Capillaria philipinensis* and *Eustrongylids excisus*), all which were found in the intestine and stomach of their fish hosts ; while *B. bayad* accounted for 207 (53.21%) of the total parasites, *B. docmak* recorded 182 (46.79%) parasites. *B. bayad* had higher prevalence (43.33%) but lower intensity (3.98) than *B. dockmak* with 35.83% prevalence and 4.23 intensity. The haematological parameters of the infected fishes indicated reduction in the haemoglobin concentration (Hb), packed cell volume (PCV) and red blood cells (RBC). However, the infected fishes had higher white blood cell (WBC) than uninfected fishes. However, significant ($p<0.05$) existed among the studied haematological parameters of the fish samples. Biochemically, the infected samples of both species recorded higher aspartate amino transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) than uninfected counterparts. Also, creatinine and urea were higher in the infected samples of both species than uninfected ones.

Keywords: Parasites, haematological parameters, biochemical parameters, *Bagrus bayad*, *Bagrus docmak*, Lower River, Benue Nigeria

Introduction
Bagrids are freshwater fish of Africa, Southern and Eastern Asia; they are commonly found in major lakes and rivers. Bagrids are also a very diverse family ranging from *Bagrus meridionalis*, the largest fish native to Lake Malawi, to the diminutive Hyalobagrus flavus of Southeast Asia that rarely exceeds one inch (Okpasuo *et al.*, 2016) [29]. They are common in commercial catches of River Benue where they provide rich protein source in the diets of the populace. Parasites are a serious concern to freshwater and marine fishes throughout the world, and are of particular importance in the tropics (Iyaji *et al.*, 2008, Bichi and Dawaki, 2010) [16, 5] They constitute a major limiting factor to the growth of farmed fish in Nigeria (Bichi and Yelwa, 2010) [5].The effects of parasites on fish include nutrient devaluation (Hassan *et al.*, 2010), alteration of biology and behaviour (Lafferty, 2008)[21].lowering of immune capability, induction of blindness, morbidity, mortality, growth and fecundity reduction (Nmor, 2004) [27] and mechanical injuries depending on the parasite species and load (Echi *et al.*, 2009a).
Haemato-biochemical indices have been employed in effectively monitoring the responses of organisms to stressors and thus its health status under such adverse conditions. Generally, haematological tests are used to establish normal health status and to diagnose diseases caused by various factors: viz heavy metals, environmental stress, parasitic infections, genotoxic effect of pollutants, nutrition, and pollution in human and veterinary science (Fedato *et al.*, 2010) [13]. According to Caruso *et al.*, (2005) [8], haematological parameters act as physiological indicators to changing

External environments as a result of their relationship with metabolic levels (energetic), respiration (haemoglobin) and defence mechanisms. They also provide an integrated measure of the health status of an organism, which over time manifests in changes in weight (Yaji and Auta, 2007) [37] hence, the changes associated with Haemato-biochemical parameters due to various parasites establish a database, which could be used in diseases diagnosis and in guiding the implementation of the treatment or preventive measures. Several researches on the biology, ecology and parasites of the fishes of Lower River Benue have been documented (Ogbe *et al.,* 2008, Omeji *et al.,* 2015) [28, 31] among others. However, none of these studies accounts for the physiological impact of parasites on the fishes of the River. This work therefore, aimed at investigating the impact of parasites on the haematological and biochemical parameters of selected bagrid species from Lower River Benue, Makurdi, Nigeria.

Materials and methods

Sample collection and Examination of fish samples for ecto and endo parasites

240 fish samples comprising of 120 each of *Bagrus bayad* and *Bagrus docmak* of different sizes were purchased from Wadata Market, Makurdi, Benue State. The fins, gills and skins (external surfaces) of the fish samples were brushed into a petri-dish containing normal saline and examined with a hand lens for the presence of ectoparasites. Scrapings from the skin, fins and gills of each fish were taken and smeared on glass slides for examination of any parasite [14]. Fish gills were dissected out and each gill filament and arch was examined with a hand lens for the presence of any parasitic cysts. The fishes were dissected to expose the viscera. The visceral cavities and organs were examined for endoparasites. The guts were removed and placed in petri dishes and the contents of the guts were flushed with normal saline into beakers and then shaken in order to loosen mucus and other intestinal debris. After centrifugation and decanting of the supernatant, parasites were recovered from the residue. Recovered parasites were mounted on slides and viewed under light microscope and identified to species level using appropriate keys by Yamaguti (1963) [31]. All recovered parasites were counted and recorded.

Haematological and biochemical assay of the fish samples

From the 240 fish samples that were bought, blood samples were collected from the caudal peduncle and heart of the gently sacrificed fish samples using 2ml plastic syringe and needle treated with anti-coagulant as described by (Lucky, 1977) [23]. The blood samples for haematological studies were preserved in EDTA embedded bottles while that of enzymes; Aspartate amino transaminase (AST), Alanine aminotransferase (ALT) and alkaline phosphatase (ALP), Creatinine and Urea analysis were preserved in heparinised bottles. Packed cell volume (PCV) was determined using Hawsley micropillary tubes and centrifuged for 5 minutes (Abudu, 1994) [1]. Red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb) and packed cell volume (PCV) were analysed according to the methods of (Blaxhall, 1973) [7]. The heparinised blood samples were centrifuged at 300 rpm for 10 minutes and the serum collected for analysis. AST and ALT, ALP, urea and creatinine were analysed according to the methods of Jenkins (2004).

Statistical analysis

Data obtained were subjected to statistical analysis using one way analysis of variance at 95% probability and means separated using Fishers Least Significant difference test at 95% probability.

Results

Results of the parasite prevalence and intensity of *Bagrus bayad* and *Bagrus docmak* used for this study are shown in Table 1. Out of the 240 fish samples comprising of 120 each of *Bagrus bayad* and *Bagrus docmak* used, 145 (60.42%) fish samples comprising of 67 (46.21%) samples of *Bagrus bayad* and 78 (53.79%) samples of *Bagrus ducmack* were not infested with any parasite while 95 (39.58%) fish samples comprising of 52 (54.74%) samples of *Bagrus bayad* and 43 (45.26%) samples of *Bagrus docmak* were infested with 389 different parasites belonging to one species of protozoan (Tricodina acuta) found on the gills and skin of their fish hosts, two species of Cestode (*Diphilobothrium latum* and *Hymenolepis nana*), two species of Nematode (*Capillaria philipinensis* and *Eustrongylids excisus*), all which were found in the intestine and stomach of their fish hosts ; while *Bagrus bayad* accounted for 207 (53.21%) of the total parasites, *Bagrus docmak* recorded 182 (46.79%) parasites.
Bagrus bayad had higher prevalence (43.33%) but lower intensity (3.98) than *Bagrus dockmak* with 35.83% prevalence and 4.23 intensity, respectively.

Fish species	No. of fish examined	No. (%) of fish infested	No. (%) of parasites recovered	No. (%) of fish not infested	Parasitic Prevalence	Parasitic intensity
B. bayad	120	52 (54.74)	207 (53.21)	67 (46.21)	43.33	3.98
B. docmak	120	43 (45.26)	182 (46.79)	78 (53.79)	35.83	4.23
Total	240	95 (39.58	389 (100)	145 (100)		

Results of the mean haemo-biochemical parameters of *Bagrus bayad* and *Bagrus docmak* used for the study are shown in Table 2. Haematological parameters such as red blood cell (RBC) of 5.76±0.93 and packed cell volume (PCV) of 31.58±3.94 were higher in *B. docmak* than *B. bayad* with lower RBC and PCV of 5.74±0.97 and 31.23±3.83, respectively. Conversely, Haemoglobin concentration (Hb) of 9.21±1.27 and white blood cell of 4.38±1.83 were higher in *B. bayad* than *B. docmak* with mean Hb and WBC of 9.21±1.27 and 4.25±1.75, respectively. Biochemically, while the mean alkaline phosphatase (ALP) of 64.76±6.50, creatinine (1.34±0.17), and Urea (31.73±3.92) were higher in *B. bayad* than *B. docmak*, mean aspartate amino transaminase (AST) of 84.67±6.36 and alanine aminotransferase (ALT) of 112.22±10.04 were higher in *B. docmak* than *B. bayad*

Mean haemato logical and biochemical parameters of *B. bayad* and *B. docmak* from Lower River Benue

Parameters	Fish species		P-value
	B. bayad	***B. docmak***	
RBC (Cells/mm^3)	5.74±0.97	5.76±0.93	0.99
PCV (%)	31.23±3.83	31.58±3.94	0.95
HB (g/dl)	9.21±1.27	8.99±1.10	0.90
WBC (Cells/mm^3)	4.38±1.83	4.25±1.75	0.96
AST (U/L)	84.54±6.57	84.67±6.36	0.99
ALT (U/L)	111.69±10.43	112.22±10.04	0.97
ALP (U/L)	64.76±6.50	64.62±4.31	0.98
CRT (U/L)	1.34±0.17	1.33±0.19	0.99
UREA (U/L)	31.73±3.92	31.39±2.53	0.90

Note: RBC = Red Blood Cell, PCV = Packed Cell Volume,
Hb = Haemoglbin Concentration,

WBC = White Blood Cell, AST = aspartate amino transaminase, ALT = alanine aminotransferase, ALP = alkaline phosphatase, CRT = Creatinine

Results

of the mean haemological and biochemical parameters of infected and uninfected *B. bayad* and *B. docmak* used for the study are shown in Table 3Haematologically, RBC, Hb and WBC were higher in the infected samples of both species than the uninfected counterparts. Nonetheless, it was observed that the mean WBC was higher in the infected *B. bayad* ($1.21\pm0.01 \times 10^2$) and *B. docmak* ($1.22\pm0.01 \times 10^2$) than the uninfected samples of *B. bayad* ($7.55\pm0.05 \times 10^1$) and *B. docmak* ($7.29\pm0.29 \times 10^1$), respectively. However, there was significant difference ($p<0.05$) in the haematological parameters of infected and uninfected samples of fish species used for the study.

Biochemically, infected samples of both species recorded higher AST, ALT, ALP, Creatinine and Urea than the uninfected counterparts of the fish species.

Mean haemological and biochemical parameters of infected and uninfected *B. bayad* and *B. docmak* from Lower River Benue

Parameters	Fish species/health status					
	B. bayad		P-value	***B. docmak***		P-value
	Infected	**Uninfected**		**Infected**	**uninfected**	
RBC (Cells/mm^3)	4.08±0.08	7.40±0.04	0.01	4.17±0.01	7.35±0.35	0.01
PCV (%)	24.60±0.01	37.86±0.30	0.00	24.77±0.02	38.39±0.61	0.00
HB (g/dl)	7.04±0.01	11.38±0.52	0.01	7.09±0.01	10.89±0.14	0.01
WBC (Cells/mm^3)	$1.21\pm0.01 \times 10^2$	$7.55\pm0.05 \times 10^1$	0.00	$1.22\pm0.01 \times 10^2$	$7.29\pm0.29 \times 10^1$	0.02
AST (U/L)	95.93±0.08	73.16±0.03	0.00	95.68±0.33	73.66±0.35	0.00
ALT (U/L)	129.75±0.25	93.64±0.37	0.00	129.60±0.50	94.85±0.26	0.00
ALP (U/L)	76.01±0.09	53.52±0.49	0.00	76.00±1.00	52.96±0.05	0.00
CRT (U/L)	1.64±0.02	1.04±0.02	0.00	1.66±0.05	1.33±0.19	0.01
UREA (U/L)	38.50±0.50	24.96±0.34	0.00	37.60±0.60	24.49±0.04	0.00

Discussion

Haematological parameters are important in diagnosisof the functional status of the fish infested with parasites (Joshi *et al.* 2002). They are also used to evaluate the physiological condition and nutritional state of fish (Chagas *et al.* 2003) Blood as one of the haematological parameters use to evaluate the health status of fish, is a good bio-indicator of the health of an organism (Joshi *et al.* 2002). It also acts as a pathological reflector of the whole body. There was significant variation ($p<0.05$) in the haematological parameters of the studied infected and uninfected fish samples during the study period. This variation could be due to the physiological state and intensity of infection of the host fish. This observation is in line with the reported wok of Abdul *et al,* (2009). Also, a well marked increase in eosinophils was observed in all the helminth-infected fish samples. The haematological manifestation of the infected fish is suggestive of *anaemia* and the *eosinophilia* may be believed to be associated with defensive and immunological responses of the fish (Ugbor, *et al* 2015). Marked reduction in the RBC, Hb and PCV from fishes infected with hemoparasites had been reported (Shahi *et al* 2013) [13] The present study showed that parasitic infection alters haematological parameters of *B. bayad* and *B. docmak*. Parasites caused reduction in RBCs, Hb and Hct. some studies demonstrated a reduction in RBC, Hb and Hct in relation to parasitism (Martins *et al*. 2004) [25]. Nevertheless, other studies showed that parasites as a stressor stimulate the primary stages of stress and affect haematocrit. Parasitic infection stimulates releasing catecholamine, which can mobilize red blood cells from spleen or induce red blood cell swelling as a result of fluid shift into the intracellular compartment (Wells and Webber 1990) [35]. WBCs play a great role during parasitic infestation by stimulating the haemopoietic tissues and the immune system by producing antibodies and chemical substances working as defense against infection (Lebelo *et al.* 2001) [21]. The values of WBCs were higher in infected fish compared to uninfected samples. The increase of serum WBCs has been well known as immune response to variety of infections. Therefore, the increase or elevation of WBCs in infected samples B. bayad and B. docmak may be a response of cellular immune system to parasitic infection. The activity of the serum ALP in the parasite infected bagrid species of the Lower River Benue showed a significant increase when compared to the uninfected fishes. This finding is in agreement with the observations of (Ahmed, 2006) and (Witthwaskid, *et al*. 2003) [36] who reported increase in ALP in Fasciola infected monkey. The authors reported that the elevation in the ALP activity may be due to the parasite penetration of the into the bile duct. Activities of AST and ALT were significantly

higher ($P<0.05$) in the infected fishes when compared to the uninfected fishes. A similar result was observed by (Younis, 1999) who reported that AST, ALT and Urea showed significant increase in *O. niloticus* infected with external protozoa and monogenetic trematodes. Osman *et al*. (2009) reported that blood serum AST, ALT enzyme activities, creatinine and Urea values were increased in Trichodina infected *Clarias gariepinus*. Mahmoud *et al*. (2011) [24] reported that the blood serum AST, ALT, creatinine and urea values were elevated in the infected *O. niloticus* and *C. gariepinus*. The increase in the activity of AST and ALT in the serum of the infected fishes revealed that the parasites had effect on the parenchymous tissues and skeletal musculature (Egbu 2011), which probably may have altered the permeability (Kabir, 2009) and integrity of cell organelles as reported by (Adamu and Iloba, 2008) The observed sequential pattern of ALT and AST activities in this study are biochemical indications leading to liver cytolysis, indicating structure disturbance and integrity of the cell system (Dere, 2001). Alterations in the activities of of ALT and AST may have harmful effects on the amino acid metabolism of the tissues and consequently the intermediates that are required for gluconeogenesis (Kabir, 2009). The elevated of serum urea and creatinine levels in this work could be attributed to the parasitic infestation of the fish samples. Kabir and Ovie (2011) [12] reported that creatinine leaves the muscles and enters the blood where it is a waste product largely from the muscle breakdown. It is removed by filtration through the glomeruli of the kidney and excreted as urine. The increase in the level of creatinine in the infected fishes may be as a result of the alteration of the muscles structure of the infected fishes by parasites (Egbu, 2011) [12]. Mahmoud *et al.* (2011) also reported elevated levels of creatinine and urea in fishes infected with protozoan parasites. Urea is a major byproduct of protein catabolism. The increases in the urea content in the infected fishes of this study could be as a result of the gill dysfunction caused by protozoan infestation in the gills, as urea are mainly excreted through the gill (Murray *et al*, 1990). Also, increased in the urea content in the infected fishes may be due to the inflammatory reactions of intoxication produced by the parasites in the infected fishes. Mahmoud *et al.* (2011) [24] had made similar observation.

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