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HAEMATOLOGICAL CHANGES OF *Clarias gariepinus* JUVENILES FED DIFFERENT DIETARY LIPID.

Oshoke J.O. *[1], Olukunle O.A. [2], Ajayi, A.I. [4], Dasuki A. [1] and Saulawa L.A. [3]
[1]. Department of Fisheries and Aquaculture, Federal University, Dutsin-Ma, Katsina State.
[2]. Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Oyo State.
[3]. Department of Animal Production and Health, Federal University, Dutsin-Ma, Katsina State.
[4]. Department of Chemistry, University of Ibadan, Ibadan, Oyo State.
*Correspondence author E-mail: mailtina04@yahoo.com or joshoke@fudutsinma.edu.ng

ABSTRACT
Twelve weeks feeding trial was conducted to determine the effects of different dietary lipid sources on the hematological changed in *Clarias gariepinus* juveniles. 6 iso-nitroginous diets were formulated at 45% CP and fed to triplicate groups of 15 juveniles. The feed contained (Palm Seed Oil (PSO), Ugwu Seed (USO), Soya Bean Oil (SBO), Almond Seed Oil (ASO), Mixture Of All the vegetable oil + the fish oil (MOA) and Cod Liver Oil (CLO) which is the control. The oils were added at 5% inclusion level respectively. Fish of mean weight 22.83 ± 0.30g were fed these experimental diets in triplicate groups. The hematological analyses of fish showed that red blood cell, white blood cell, Erythrocyte sedimentation rate (ESR), Mean cell volume (MCV), Mean cell Haemoglobin (MCH) and packed cell volume were not significantly different ($p<0.05$), but haemoglobin concentration and Mean cell haemologlobin concentration (MCHC) were significantly different ($p>0.05$). The present study showed that PSO, USO, ASO, SBO and MOA can effectively replace cod liver oil without compromising the health of African catfish, *Clarias gariepinus*.
Key words: Dietary lipid, Juveniles, Haematology, *Clarias gariepinus*

INTRODUCTION
Global catches from the feed-grade fisheries that provide fish oil (FO) and fish meal for aqua feed formulations have reached their sustainable limits (Pike& Barlow,2002) and it is likely that within a decade or so there may be insufficient FO to meet the quantities required for current aquaculture growth (Tacon ,2004). Consequently, there has been considerable interest in introducing sustainable alternatives to fish meal and FO that reduce reliance on marine raw materials (Tacon, 2005).
Vegetable oils are viable alternatives as they are readily available and more cost-effective compared to FO. Many studies have reported that vegetable oils can partially or fully replace FO in fish diets without compromising growth performance as long as the essential fatty acid requirements of the fish are met (Turchini,etal,2009). However, the adverse effect of feeding fish with vegetable oils particularly on haematological parameters is very scanty. Blood is a good indicator in determining the health of an organism (Joshi et al., 2002c), it also as a pathological indicator of the whole body, and hence haematological parameters are important in diagnosing the functional status of an animal exposed to suspected toxicant (Omitoyin, 2006).
This study therefore investigates the changes in haematological parameters of juvenile catfish fed with different dietarylipid.

MATERIALS AND METHODS
The experimental work was carried out in the research laboratory of the Department of Wildlife and Fisheries Management teaching laboratory, University of Ibadan, Nigeria. A total of eighteen plastic circular experimental tanks of 45 litres (30 cm depth, 36 cm width and 52 cm length) covered with mosquito mesh nylon screen to prevent fish from jumping out and possible predation were used. Each of the six treatments was replicated in triplicates. Juveniles of the African catfish, *Clarias gariepinus* were obtained from a local fish hatchery and transported in oxygen bags to the laboratory. The fish were then acclimatized to laboratory conditions and fed with a commercial fish feed (35% CP) for 14 day. After acclimation, groups of fifteen *Clarias gariepinus* juveniles (mean weight 22.82 ± 0.30g) were randomly stocked into the eighteen circular plastic tanks containing 30litres of water each for the growth trials. Experimental tanks were well aerated using air stones and aerator pump (Lawson, 1995) throughout the period of the experiment to maintain relatively uniform physiochemical parameters.
Each of the diets was fed to the fish in triplicate at 5% body weight twice daily (between 8.30am and 9.00am, and 5.30pm and 6.00pm) for 84days. The weight of each group of fish was taken fortnightly using electronic top loading balance and the feed adjusted accordingly.
The water quality parameters of dissolved oxygen, temperature and pH were monitored on alternate days. Early in the morning (7.00 – 8.00 am) on the days when the water quality parameters were taken. Digital dissolved metre (manufactured by American Marine Inc.) was used to take the Dissolved oxygen, while the water temperature and

pH values of the experimental tanks were measured using Digital/electronic temperature probe and a pH meter respectively(Table 3). Fish meal, mineral/vitamin premix, soya bean meal, yellow maize, salt and binder used in this experiment/study were obtained from a feed miller in Ibadan, Nigeria. The Ugwu (*Telfaira occidentalis*) seeds were bought from Ojoo market and the oil from the seed was extracted using continuous soxhlet extraction technique with hexane. The almond seeds (*Terminalia catappa*) were picked from trees within the premised of University of Ibadan, Nigeria. The seeds were shelled by cracking to remove the kernels inside. The kernels were ground to powder in a hammer mill and the oil from the seed was extracted using the continuous soxhlet extraction technique with hexane. The palm nuts were also bought from Ojoo market and the oil from the seeds extracted locally. The soya bean oil was obtain from the market while cod liver oil, which is the control was obtained from the University of Ibadan pharmacy.

All dry ingredients were milled together with the hammer milling machine to obtain fine particulates. The crude protein content of the diets were kept essentially at the 45% level since this was determined as the protein requirement of juveniles catfish hybrid in a previous experiment (Eyo and Falayi, 1999) Each diet was first mixed dry and later with just enough warm water to obtain homogenous hard-paste(dough) and pelletized out into flat tray through 2mm die disc holes in different lengths.

Using the ingredients, six practical diets containing 45% crude protein, each having different lipid sources was formulated respectively. Diet CLO (control diet) contains cod liver oil, Diets PSO, contains palm oil, Diet ASO contains almond seed oil, Diet MOA contains a mixture of all the vegetable oil + fish oil, Diet USO contains ugwu seed oil and Diet SBO contains soya bean oil. The pellets were sun dried at ambient temperature of 30°C for three days and stored in air tight plastic at 26°C (Table 1).

Haematological evaluation

One and a half milliliters (1½ ml) of blood were collected at the beginning of the feeding trial (week 0) and at the end of trial (week 12) from the caudal peduncle of both the test and control fish. The fish from which blood for haematology was collected, were anaesthetized with 150mg/l solution of tricaine methaine sulphonate (MS-222, Sigma Chemical co. St. Louis, MO, USA) (Wegner et. al.,1997). Blood samples were taken with 2 ml heparinized syringes and 21swg needles from the caudal vein of the fish from each treatment and put separately in 2ml heparinized tubes and taken to the laboratory for determination of Haematocrit (Hct), Haemoglobin (Hb), Erythrocyte SedimentationRrate (ESR), White Blood Cell (WBC) and Red Blood Cell (RBC) using the method of Svobodova et at. (1991).The haematological indices of Mean Cell Heamoglobin Concentration (MCHC), Mean Cell Haemoglobin (MCH) and Mean Cell Volume (MCV) were calculated (Dacie and Lewis, 2001, Joshi et al 2002a).Data collected from the experiment were subjected to one way analysis of variance (ANOVA) to test the significance of variations between the means and Least Significance difference (LSD) was used to determine the level of significance ($p<0.05$) among treatments.

RESULTS AND DISCUSSION

Result of analysis of the hematological parameters of *Clarias gariepinus* in this study showed significant difference between the treatment values. PCV value obtained showed that CLO had the highest value. The haemoglobin (Hb) values are much higher than thos obtained by Subhadra et al., (2006) for the largemouth bass with diets containing canola oil, chicken oil and menhaden fish oil, which ranged between 3.7-3.9 g dl^{-1}. Hb value of 7.20g dl^{-1} for the initial (pre-treatment) *Clarias gariepinus* and the mean values of 7.67 ± 1.23 and 7.07 ± 0.81 obtained for fish raised on diet (CLO, PSO) were similar to the mean Hb values of 7.44% obtained by Etim et. at., (1999) for *Chrysichthys nigrodigitatus* showing that the oxygen carrying abilities of the blood of these two catfishes are similar. The difference may be due to the fact that we used different species and the ability to utilize n-6 fatty acids presents in the vegetable oils differs from species to species. The value obtained in the study was within the recommended range value of 4.1 – 10.3 by Blaxhall and Daisley, 1973 for healthy fish. The con of Hb, WBC and RBC in fish fed diet MOA (mixture of all the oil), show clearly that the n-3:n-6 PUFA balance seems critical in the diet of the African catfish.

There were no significant difference in ESR, RBC, WBC ($p>0.05$) among the treatment. The value of the RBC ranged from $2.30 \pm 0.68 \times 10^{12/L}$ to $1.45 \pm 0.22 \times 10^{12/L}$ are similar to those obtained by Osuigwe et.al., (2004) for *Clarias gariepinus*. The mean cell haemoglobin concentration (MCHC) differed between treatments and did not follow a clearly defined trend. Fish fed on diet USO showed significantly lower MCHC than fish raised on all the other treatments. Lie et. al., (1989), reported that an increase in MCHC and MCH values reflect a preserving mechanism in rainbow trout activated at reduced water temperatures. There was no temperature variation in this study, hence no increase relative to the initial MCHC and MCH values were observed.

CONCLUSION AND RECOMMENDATION

In conclusion the present study revealed no inhibition to the formation of skeletal tissues, cell formation, blood formation and flow; hence the utilization of PSO, USO, ASO, SBO and MOA in the diet of *Clarias gariepinus* should be encouraged.

Table 1: Proximate Composition of diets based with different lipid source for the African catfish. *Clarias gariepinus* (% dry matter).

Ingredients	Diet PSO	Diet USO	Diet SBO	Diet ASO	Diet CLO	Diet MOA
Crude Protein (%)	46.87	45.68	46.65	45.92	45.82	46.66
Crude Fat (%)	6.79	5.64	6.12	5.98	6.87	6.48
Crude Fibre (%)	3.55	3.66	3.86	3.78	3.58	3.92
Ash (%)	16.94	16.89	17.23	15.89	17.49	17.86
Moisture (%)	6.82	7.62	8.14	8.21	6.73	7.56
NFE (%)	19.03	21.54	17.00	20.22	20.51	12.52

Table 2: Haematological parameters of African catfish *clarias gariepinus* juveniles fed with the experimental diets.

PARAMETERS	INITIAL	PSO	USO	SBO	ASO	CLO	MOA
PCV (%)	21.00	22.00 ±2.65^{a}	20.33±3.51^{a}	18.67±1.15^{a}	21.67±4.04^{a}	24.00±3.61^{a}	22.0±2.650^{a}
Hb (gm%)	7.20	7.07±0.81^{b}	3.00±3.51^{c}	5.57±0.38^{c}	6.90±1.31^{d}	7.67±1.24^{a}	7.40±1.06^{e}
RBC ($\times 10^{12}$/L)	1.84	1.79±0.46^{a}	1.59±0.12^{a}	1.45±0.22^{a}	1.95±0.56^{a}	2.03±0.68^{a}	1.85±0.52^{a}
WBC ($\times 10^{9}$/L)	16,500	17116.67^{a} ±1733.73	19150.00^{a} ±975.96	15600^{a} ±4850.78	19966.67^{a} ±7823.26	19933.33^{a} ±1527.53	19550.00^{a} ±7590.62
ESR (mm/hr)	0.20	2.00±1.00^{a}	1.33±0.58^{a}	1.33±0.58^{a}	1.67±0.58^{a}	1.67±1.15^{a}	1.67±0.58^{a}
MCV (FL)	114.13	125.94±16.61^{a}	129.15±29.01^{a}	131.30±24.28^{a}	115.15±29.61^{a}	124.86 ±30.14^{a}	122.54±19.29^{a}
MCH (Pg)	39.13	40.53±6.03^{a}	36.83±9.53^{a}	39.19±7.59^{a}	36.67 ±9.52^{a}	39.79±9.23^{a}	41.49±9.72^{a}
MCHC (%)	34.29	32.14±0.79^{a}	28.41±1.51^{abcd}	29.81±0.32^{c}	31.83±0.41^{c}	31.91±0.37^{d}	33.65±3.16^{b}
Platelet	294000.00 ±28095.08	137333.33^{a} ±118643.72	216333.33^{a} ±29871.95	131333.33^{a} ±70500.59	159666.67^{a} ±137012.16	246333.33^{a} ±36501.14	127333.33^{a}

a,b,c.... Means in the same row having different superscripts are significantly different ($P < 0.05$), while means in the same row having same superscript are not significantly different ($P>0.05$). Values given in mean ± standard deviation of three replicates.

Table 2: Mean bi-weekly water parameters of the experimental tanks

Treatments	Parameters	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
PSO	Temp (°C)	26.02±0.05	25.03±0.45	26.03±0.00	25.70±0.00	25.20±0.16	26.00±0.01
	DO (mg/l)	6.60±0.00	6.48±0.01	6.70±0.05	6.58±0.01	6.60±0.03	6.60±0.00
	PH	7.20±0.05	6.88±0.01	7.20±0.02	6.93±0.07	6.90±0.06	7.10±0.03
USO	Temp (°C)	25.80±0.01	25.10±0.08	26.01±0.01	25.10±0.04	25.27±0.12	26.00±0.02
	DO (mg/l)	6.50±0.02	6.56±0.05	6.55±0.02	6.70±0.50	6.61±0.01	6.52±0.01
	PH	6.95±0.00	6.84±0.03	7.10±0.21	6.95±0.05	6.90±0.01	7.10±0.02
SBO	Temp (°C)	25.70±0.01	24.03±0.05	25.90±0.01	25.20±0.01	24.53±0.75	25.70±0.03
	DO (mg/l)	6.46±0.02	6.55±0.02	6.47±0.01	6.70±0.01	6.39±0.04	6.47±0.02
	PH	6.90±0.08	6.84±0.01	7.02±0.01	6.90±0.03	6.78±0.01	6.95±0.05
ASO	Temp (°C)	26.00±0.00	24.27±0.38	26.00±0.02	25.80±0.03	25.17±0.00	26.05±0.01
	DO (mg/l)	6.51±0.02	6.48±0.03	6.51±0.03	6.48±0.02	6.50±0.08	6.55±0.05
	PH	6.95±0.01	6.80±0.02	7.10±0.01	6.95±0.08	6.90±0.02	7.10±0.02
MOA	Temp (°C)	25.60±0.01	24.20±0.16	25.01±0.00	25.27±0.02	24.20±0.05	26.01±0.50
	DO (mg/l)	6.45±0.02	6.12±0.04	6.44±0.01	6.42±0.01	6.04±0.02	6.52±0.04
	PH	6.84±0.01	7.10±0.21	6.90±0.06	6.84±0.01	6.90±0.08	6.88±0.01
CLO	Temp (°C)	25.70±0.01	24.90±0.08	25.80±0.02	24.90±0.00	25.10±0.08	25.80±0.01
	DO (mg/l)	6.48±0.02	6.82±0.01	6.49±0.02	6.82±0.04	6.59±0.02	6.49±0.01
	PH	7.00±0.08	6.87±0.03	7.00±0.03	6.95±0.05	6.89±0.01	7.01±0.01

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