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Maxigrain® enzyme supplementation effect on serological indices of African catfish *Clarias gariepinus* (Burchell, 1822) fed *Gliricidia sepium*(Jacq.)leaf meal

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Abstract

A total of 50 African catfish (*Clarias gariepinus)* juvenile with average weight 208 ± 6g serum indices were used to monitor the effect of levels of Maxigrain in catfish fed *Gliricidia sepium* leaf meal. Five experimental diets were formulated which is ascribe to T1, T2, T3, T4 and T5 at 0, 5, 10, 15, and 20% Maxigrain supplemented with 10% *Gliricidia sepium.* Although results showed that after twelve (12) weeks of feeding, fish fed with 15% Maxigrain (T4) supplemented with 10% *G. sepium* leaf meal gave the best response in terms of the variables monitored, all the parameters were within the normal physiological range. Hence catfish can be fed with plant protein sources with enzyme supplementation while still maintain their normal health status.

Keywords: catfish, *Gliricidia sepium*, leaf meal, Maxigrain, serology

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Introduction

High cost of feed input is a major problem to fish farmers in Nigeria and fish feed is general considered as the most expensive item in intensive fish farming. According to NRC (1993) fish feed constituents among which there is fish meal is about 40-60% of the recurrent cost of most intensive fish farming ventures, and can sometimes negate the economic viability of a farm if suitable alternative feed are not used(Bello *et al*., 2014). As a result, there is a need to look resourcefully, outwards to explore, identify and utilize other resources which are cheaper and less competitive. Various attempts have been made to reduce this high cost of fish by unconventional fish feed stuffs. Fish feed stuffs of carbohydrate and protein base have been investigated through the understanding of their chemical composition (West *et al*.,1988)Similarly, researchers obtained a range of results when they replaced the animal protein in tilapia feeds with vegetable protein specially both on economic and ethical ground(Wee and Wang, 1989) Likewise catfish has also been fed with leaf meals (Olaniyi *et al*., 2013; Ozovehe,2013; Bello *et al*.,2014).A number of plants continue to be investigated for their potential in supplementing or even replacing fish meal. *Gliricidia* has been identified to hold the potential to make contribution to fish nutrition with the possibility to reduce the total dependence of fish farming on fish meal in several locations in Nigeria. *G. sepium* is a multipurpose tree legume that is second only to *Leuceccanea leucocephala*in worldwide popularity. *Gliricidia* possess the ability to provide large qualities of high quality forage matter all year round as well as ability to maintain a sustainable environment through nitrogen fixation thus replenishing the soil (Chadhokar and Kantharaju, 1985). The leaves of *G. sepium* have a high feeding value, with crude protein comprising 20 – 30% of dry matter, a crude fibre content of about 15% and *in vitro* dry matter digestibility of 60–65% (Adejumo and Ademosun,1985; Kabi and Lukatome, 2013). Its leaves are good source of protein and needs to be examined in detail on its suitability (Dasilva and Anderson, 1995; olaniyi *et al*., 2013). At the same time, the leaves also contain anti-nutritional factors like cell wall constituents (Odo *et al*.,2016;Ogungbesan *et al*., 2016[a] ;Ogungbesan *et al*., 2016[b]; Ogungbesan *et al*., 2016[c]) condensed tannins, saponins and cyanogenic glycoside (Ogungbesan *et al*., 2016[a]). This can elicit toxicological response especially in monogastrics animal (catfish inclusive).

The use of haematological techniques in fish culture is of growing importance to toxicological research, environmental monitoring and fish health conditions (Kaneko *et al*., 2008). Fish are

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so intimately associated with the aqueous environment, often physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in the fish. (Olusegun and Adedayo, 2014).Blood is a good indicator to determine the health of an organism (Erhunmunse and Ainerua,2013). It also act as a pathological reflector of the whole body, hence haematological parameters are important in diagnosing the functional status of animals exposed to toxicants (Owolabi,2011). The addition of exogenous enzymes to feeds that contain this alternative energy and protein sources could be a potential tool for improving feed efficiency and thus increase the use of low cost, high fiber and proteinaceous feedstuffs used in compounding feed for fish have been reported by(Yasaemin and Funda 2010; Agbabiaka, *et al.,*2012;Olapade*et al.,* 2015). There has been study on levels of enzymes on performance of fish. It is against this backdrop that this experiment sets out to investigate the effect of enzyme levels on blood profile of catfish.

Materials and Methods

The study was conducted at Fisheries Laboratory of College of Agricultural Sciences. Olabisi Onabanjo University, Yewa campus, Aiyetoro, Ogun state. Nigeria.

Ingredients and diets preparation

Maize, soybean, fish meal, bone meal, wheat offal, blood meal, premix, methionine, lysine, salt, vitamin C, starch, enzymes and *Gliricidia*leaf meal. *G. sepium* leaf was plucked from their branches on the trees compound of College of Agricultural Sciences, OlabisiOnabanjo University, Yewa campus, Ayetoro, Ogun state. The leaves were sundried for 7days. All ingredients were ground into powdery form using a corn miller machine.

Table 1: Ingredient and chemical composition of experimental *Clarias Gariepinus* diet containing *Gliricidia Sepium* leaf mealwith different maxigrain levels

Feed Ingredient	Diet1 (Maxigrain enzyme 0%)	Diet2 (Maxigrain enzyme 5%)	Diet3 (Maxigrain enzyme 10%)	Diet4 (Maxigrain enzyme 15%)	Diet5 (Maxigrain enzyme 20%)
Maize	25	25	25	25	25
Soyabean meal	32	32	32	32	32
Gliricidia leaf meal	10	10	10	10	10
Fish meal	10	10	10	10	10
Bone meal	1.5	1.5	1.5	1.5	1.5
Wheat offal	10	10	10	10	10
Blood meal	10	10	10	10	10
Premix	0.50	0.50	0.50	0.50	0.50
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin c	0.05	0.05	0.05	0.05	0.05
Enzymes	0	50	100	150	200
Total	100	100	100	100	100
Crude protein (%)	40	40	40	40	40
Energy	2731.5	2731.5	2731.5	2731.5	2731.5
Crude fiber	4.511	4.511	4.511	4.511	4.511
Either extract	3.538	3.538	3.538	3.538	3.538
Ash	4.366	4.366	4.366	4.366	4.366
NFE	52.42	52.42	52.42	52.42	52.42

Five diets containing *Gliricidia* (Dry matter (%) 31.82,Crude protein (%) 21.28, Crude fibre (%) 20.76, Ether extract (%) 3.98, Ash (%) 9.16 Nitrogen free extract (%) 44.82[13]leaves were harvested, air dried for several days and subsequently milled to obtain *Glricidia* leaf meal(GLM) and incorporated into five fish diets in which maxigrain®(Cellulase 10000 i.u, Beta glucanase 200 i.u,, Xylanase 10000 i.u, Phytase 2500FTU) was added at 0g (control),50g ,100g,150g and 200g per tonne of diet 1, 2, 3,4 and 5 respectively) meal were prepared. The ingredients were thoroughly mixed together by hand; water was added to make the pre-mixed ingredient homogenized to dough-like paste. The diets were pelletized using a 2mm pellet press. The diets were finally dried using local dryer.

Diet 1- Containing 10% GLM without enzyme supplementation.

Diet 2- Containing 10% GLM with (50g) 5% enzyme supplementation.

Diet 3- Containing 10% GLM with (100g) 10% enzyme supplementation.

Diet 4- Containing 10% GLM with (150g) 15% enzyme supplementation.

Diet 5- Containing 10% GLM with (200g) 20% enzyme supplementation.

Experimental procedure

The experiment was carried out in rectangular plastic tanks of 0.46m × 0.44m with water volume level maintain 0.11m^3. A total of 50 African catfish (*C. gariepinus*) juvenile with average weight 208 ± 6g were randomly allotted at the rate of 10 juvenile per tank. The fish were allowed to acclimatize for 6 days prior to the start of the experiment.

Fish feeding and culture

The fish were fed twice daily between 8.00am and 4.00pm at 5% of the body weight throughout the experiment. The ration was adjusted when new weights of fish for each experimental unit were determined. Left-over feed and faeces in each tank were siphoned out every day. The water in each tank was changed every day in the morning through flow through process.

Method of collecting blood samples

Blood samples were collected at 12th week of the experiment. 2ml blood samples were collected from caudal peduncle of the fish using 2ml disposable syringe into two different containers at a time from each animal. Blood for haematological indices was collected into bijou bottles containing Disodium salt of Ethylene diamine tetraacetic acid (EDTA) as anticoagulant, while blood for serum biochemical and chemical profile was collected into universal bottles, and. allowed to clot at room temperature and the serum separated by centrifugation at 2, 300 rpm for 10min. All samples were kept in the refrigerator and later taken for analysis.

Analysis of blood indices

Thetotal protein (T.prot) was determined using the biuret method (Reinhold, 1953), albumin (Alb) concentration was estimated by the methods of (Doumas *et al*., 1971). The globulin (Glob) concentration was calculated by subtracting Alb concentration from total protein concentration. Glucose (Glu) was estimated by the method of (Trinder, 1969) while Triglyceride (Trigly) was determined through the procedures of Rice (1970).

Blood cell count

Electronic particle counter was used to obtain the total erythrocyte (RBC) and leukocyte (WBC) counts and its components. Haemoglobin concentration (Hb) was determined by cyanmethemoglobin using a coulter haemoglobinometer. The blood cells were counted on the counting chamber of haemocytometer with the aid of compound microscope:

- RBC: No of cells counted x3x10x200 (10^6 mm^3)
- WBC: No of cells counted x0x25x10x20 (10^4 mm^3)

Haemoglobin estimation

Haemoglobin meter was used for haemoglobin estimation based on acid haematin method:

$$\text{Haemoglobin} = \frac{\text{Value obtained}}{100} \times 17.2 \text{ mg/100mL}$$

Packed cell volume

The packed cell volume was measured after placing sealed micro-haematocrit tube in a centrifuge at 10,500 rpm using micro-haematocrit reader and expressed as percentage.

Mean corpuscular volume (MCV): MCV was calculated from the haematocrit value (PCV, % and the Erythrocyte count (Er mm^3):

$$MCV\ (\mu^3) = \frac{PCV}{Er} \times 10$$

Mean corpuscular haemoglobin concentration (MCHC): This was obtained using the formula:

$$MCHC\ (\%) = \frac{Hb}{PCV} \times 100$$

Mean corpuscular haemoglobin (MCH): This was expressed in picograms (ρg):

$$MCH\ (pg) = \frac{Hb}{Er} \times 10^2$$

Statistical analyses

Data was subjected to analysis of variance (ANOVA) using completely randomized design. Mean was analysed for significance difference using Least Significance (P<0.05) difference according to the procedures of statistical analysis system (S.A.S.2002)

Results and Discussion

Table 2 shows the PCVor hematocrit range from T1 0% (32.12±0.23) to T4 15%(47.03±0.12). Low PCV can lead to heamodilution and also potentiate anaemia (Bell *et al*., 1972) This ranges falls between the normal ranges of 20 – 50 as reported by(Pietse *et al*.,1981)The RBC or Erythrocytes (x10^{12}/L) was highest in T4 15% (3.00±0.03) and lowest in T1 0%(1.90±0.03)respectively. Table 3 shows there is significant (P<0.05) different which means the best performance is from T4 15% (3.00±0.03) and that the Maxigrain released a lot of nutrient for growth and development heamopoiesis inclusive (Ogungbesan *et al*., 2016[c]). Range recorded in this study is fairly comparable to 1.70 – 4.00 recorded by (Dienye and Olumumi,2014).The increase in RBC may be ascribed to high concentration of metabolitesin the diet but erythrocyte count is greater than one high and indicative of high oxygen carrying capacity of the blood which is characteristic of fishes capable of aerial and with high metabolic activity (Lenfant and Johansen,1972).The haemoglobin Hb(mmo/L) range was highest in T4 15% (2.20±0.01) and lowest in T1 0% (1.67±0.92) as recorded in Table 3. These are high and fell within the range of 5.6-15.8g/dl(0.868-2.449 (mmo/L)) reported by (Mulcahy 1970).The increase in the level of Maxigrain led to increase in the value of haemoglobin which means there is a possible effect on the haemoglobin concentration to some extent.WBC(×10^6/L)Leucocytes was lowest in T1 0%(15.50±1.10) and was highest in T5 20%(24.11±1.13) as recorded in Table 3, while T3 10%(21.30±1.78) was next to T5 20%.

Table 2: The effect of Maxigrain® levels on haematological parameters of *Clarias gariepinus* fed *Gliricidia sepium* leaf meal

Parameters	**Diet1** (Maxigrain enzyme 0%)	**Diet2** (Maxigrain enzyme 5%)	**Diet3** (Maxigrain enzyme 10%)	**Diet4** (Maxigrain enzyme 15%)	**Diet5** (Maxigrain enzyme 20%)	**Pooled SEM**	P-values	L	Q
PCV	32.12^e	40.01^d	39.03^c	47.03^a	39.11^b	1.83	0.793	ns	s
RBC(x 10^{12}/L)	1.90^a	2.61^b	2.34^d	3.00^a	2.41^c	0.04	0.856	ns	s
Hb (mmol/L)	1 .67^d	1.94^c	1.94^d	2.20^a	2.03^b	0.09	0.920	s	s
WBC (×10^6/L)	15.50^e	18.72^c	21.30^b	17.89^d	24.11^a	1.20	0.914	s	s
Neutrophil (×10^6/L)	33.10^c	32.12^d	36.01^a	34.00^b	33.33c	1.32	0.872	ns	s
Lymphocyte (×10^9/L)	66.35^b	68.15^a	63.20c	66.50^b	66.74^e	1.41	0.886	ns	s
MCV (fl)	173.70^a	163.90^c	169.52^b	156.11^d	162.56^b	11.53	0.874	ns	s
MCH (fmol)	0.88^b	0.90^a	0.84^c	0.73^e	0.79^d	2.03	0.882	s	ns
MCHC (g/L)	327.00^a	313.60d	321.50 ^{c}	302.00 ^{b}	305.00 ^{e}	18.74	0.886	s	ns

A, b, c, d and e means on the same row with different super scripts are significantly different (P<0.05); PCV: Parked Cell Volume; RBC: Red Blood Cell; Hb: Haemoglobin; WBC: White Blood Cell; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; s: significant; ns; non-significant.

Table 3: The effect of Maxigrain levels on serum biochemistry of *Clarias gariepinus* FED *Gliricidia sepium* leaf meal

Parameters	**Diet1** (Maxigrain enzyme 0%)	**Diet2** (Maxigrain enzyme 5%)	**Diet3** (Maxigrain enzyme 10%)	**Diet4** (Maxigrain enzyme 15%)	**Diet5** (Maxigrain enzyme 20%)	**Pooled SEM**	P-values	L	Q
Total protein(g/L)	24.00^{e}	35.00^{d}	38.80^{b}	41.00^{a}	36.00^{c}	1.80	0.964	s	s
Albumin (g/L)	13.00^{d}	21.00^{c}	25.20^{b}	28.00^{a}	25.10^{b}	1.52	0.912	s	s
Globulin (g/L)	11.00^{b}	14.00^{b}	13.60^{b}	13.00^{b}	11.00^{a}	1.06	0.900	ns	s
Glucose (mmol/L)	3.20	3.14	3.19	3.14	3.26	0.97	0.895	ns	s
Triglyceride (mmol/L)	5.78	5.81	5.68	5.70	5.81	0.16	0868	ns	s

a, b, c, d and e means on the same row with different superscript are significantly different (P<0.05). s: significant; ns; non-significant.

Table 4: Correlation coefficient among serum biochemical indices

Parameters	**T Prot**	**Albumin**	**Globulin**	**Glucose**	**Triglyceride**
Total protein	--------	0.9353	0.6802	0.9104	-0.5054
Albumin	0.9353	--------	0.3842	0.8302	-0.6957
Globulin	0.6802	0.3842	-------	0.6613	0.0952
Glucose	0.9104	0.8302	0.6613	--------	-0.2227
Triglyceride	-0.5054	-0.6957	0.0952	-0.2227	----------

Table 5: Correlation coefficient among haematological indices

	PCV	**RBC**	**Hb**	**WBC**	**Neut**	**Lyph**	**MCV**	**MCH**	**MCHC**
PCV	------	0.9617	0.9360	0.2737	0.2950	-0.1913	-0.6307	-0.8129	-0.7617
RBC	0.9617	-------	0.9323	0.2417	0.2015	0.0641	-0.8144	-0.9130	-0.7184
Hb	0.9360	0.9320	--------	0.4104	0.2512	-0.1053	0.2512	-0.7803	-0.6038
WBC	0.2737	0.2417	0.4104	-------	0.4353	-0.4320	-0.1963	-0.2131	-0.1362
Neut	0.2950	0.2015	0.2512	0.4353	-------	-0.8602	0.0202	-0.0921	-0.1851
Lymp	-0.1913	0.0641	-0.1053	-0.4320	-0.8602	--------	-0.1577	-0.0556	0.0727
MCV	-0.6307	-0.8144	-0.7002	-0.1963	0.0202	-0.1577	--------	-0.8907	0.4287
MCH	-0.8129	-0.9130	-0.7803	-0.2131	-0.0921	-0.0556	-0.8907	--------	0.7919
MCHC	-0.7617	-0.7184	-0.6038	-0.1362	-0.1851	0.0727	0.4287	0.7919	--------

PCV: Parked Cell Volume; RBC: Red Blood Cell; Hb: Haemoglobin; WBC: White Blood Cell; Neut: Neutrophil; Lymh: Lymphocyte; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration.

The value (lowest and highest) in this study was higher compared with 7.20 – 8.02 recorded by ((Dienye and Olumumi, 2014). High WBC count could sometimes be associated with microbial infection or the circulating system (Oyawoye and Ogunkunle, 1998). Abnormally higher (leucocytosis) and lower (leucopenia) are two undesirable conditions in that, the former is an indication of damages, destruction of tissues and infections, while the latter a signal of a situation in which the circulation and marginal pool of WBC is small and consequently reduced phagocytosis in case of infection(Ogungbesan *et al*., 2009) Neutrophil ($x10^6$/L) was highest or usually in T3 10% (36.01±2.09) and lowest in T2 5% (32.12±1.33) while Lymphocyte ($x10^9$/L) was usually highest in T2 5%(68.15±1.10) and lowest in T3 10%(63.20±1.60) as shown in Table 3. Both are differential count of leucocyte (%) and the trend is difficult to ascribe to inclusion level of Maxigrain. The trend of both is not related to inclusion level of enzymes but must not be usually low such that the system will not be able to fight against any inclement or unhealthy condition(Ogungbesan *et al*., 2016[c]) MCV(fl) was highest in T1 0% (173.73±11.43) and lowest in T4 15%(156.11±12.90) are shown in Table 3. They are however within the range of 87.50 - 210.00 recorded by Dienye and Olumumi (2014). MCH (fmol) was highest in T2 5% (0.90±0.02) and lowest in T4 15% (0.73±0.01), they are also in the range of 0.396–1.224 reported by Dienye and Olumumi (2014). MCHC (g/L) was highest in T1 0% (327.00±11.70) and lowest in T5 20%(305.00±12.20) as expressed in Table 3 above. MCHC(g/L) really gives the actual amount of haemoglobin in RBC while the abnormally high haemoglobin in plasma leads to high viscosity, low oxygen uptake, abnormal conjugation of haemoglobin and haptoglobin can lead to hyper haemoglobinuria(Ogungbesan *et al*., 2009).

Total Protein (g/L) was highest in T4 15% (41.00±3.30) and lowest in T1 0% (24.00±2.20) (Table 3). Concerning Albumin (g/L) and Globulin (g/L) the highest and lowest are in T4 0% and T1 20% (28.00±2.20 and 13.00±1.20) as well as T5 20% and T1 0% (18.00±01.20 and 13.00±1.00) with T3 10% (13.00±01.00) respectively (Table 4). Glucose (mmol/L) and Triglyceride (mmol/L) values shows no significant (P>0.05) difference among the treatment as shown in Table3 which suggest that Maxigrain can be incorporated to the level of 15% in fish although, Maxigrain is designed for grain base diet, it is also useful in forage base diet (Ogungbesan *et al*., 2009;Ogungbesan*et al*., 2016[b] ; Ogungbesan *et al*., 2016[c]).

In Table 4, Albumin an intergral part of total protein hence there is high correlation (0.9353) but not as high as that with Globulin (Glob). Protein with Glucose (Gluc) is also high thus could be due to that fact that through gluconeo-genesis or it's reversal same substrate can be used to synthesis de-novo the both biochemical parameters unlike with that of triglyceride which was negative and very low (-0.5054).(Kaneko *et al.*, 2008) . Albumin with globulin as usual are not highly correlated in that they integral part of protein as such when one has high value, the other will have lower value. (Kaneko *et al.*, 2008) Albumin and Glucose had also high correlation due to the aforementioned reason. In the same vein, Albumin with Triglyceride had negative correlation (-0.6957) Globulin has relative high correlation with Glucose but not as high as that of albumin and tremendously uncorrelated with triglyceride while Glucose and Triglyceride were also negatively correlated (Kaneko *et al.*, 2008)

PCV (Table 5) a derivation of red blood cell had high correlation with RBC and HB(RBG PCV and HB are three major variables in whole blood), with low values with Neut, Lymph, and negative with lymph, MCV, MCH and in CHC. The same trend was observed in R.B.C with HB, WBC, HCT, lymph, MCV, MCH and MCHC, RBG PCV and HB de novo synthesis are being controlled by similar factors while HCT and lymph are indication of infections and not serving the same function as the above three likewise MCV, MCH and MCHC are derivatives of interaction of the three major variable viz protein, haemoglobin and haematocrit which could either antagonistic or synergistic as such the correlation cannot be direct or high albeit it is pertinent that these MCV, MCH and MCHC are within the limit of the threshold of the fish wellbeing (Kaneko *et al.*, 2008)

Conclusion

Having gone through the haematological indices and the serological biochemistry of the experimental animals Maxigrain is commercially designed to maximised grain base diets. It is also useful in the degradation of cell wall content of leaf base diet and attenuation of plant secondary metabolites as reflected in the variables monitored above in which all the nutrients were effectively utilized in term biochemical parameters and subsequent haematopoiesis. It could be recommended that for efficient performance Maxigrain could be incorporated up to the level of 15% inclusion. It can be concluded that the inclusion (almost all) did not potentiate any haematological disorder. Research

will be recommended in future towards the palatability of such evolving product from this type of experiment.

Conflict of interest

The authors have no conflict of interest to declare.

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