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# HAEMATOLOGICAL PROFILE OF *CLARAIS GARIEPINUS* FINGERLINGS FED DIETS CONTAINING VARYING LEVELS OF ONIONS POWDER

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### Abstract

This aim of this paper amongst others is to evaluate some haematological indices and blood serum chemistry of fingerlings of *Clarias gariepinus* fed experimental diets containing onion powder (*Alium cepa*) as an additive. Five experimental diets (at various levels of inclusion of onion powder) were formulated. All experimental diets were isonitrogenous (31-32% crude protein). Fingerlings of *C. gariepinus* with initial mean weight of  $15.23\pm2.18g$  were fed *ad lib* on allotted diets twice per day at 08.00 hrs and 18.00 hrs for 60 days. The experiment was a complete randomized design. Fish fingerlings fed experimental diets showed significant (p $\leq$ 0.05) differences with respect to all the haematological indices that were evaluated. Similarly, creatinine total protein, alkaline phosphate, urea, and glucose of fingerlings fed diets containing various inclusion levels of onion powder were significantly different from the control. This study shows that while glucose levels declined with inclusion of onion powder, the status of haemoglobin, white blood cell count increased.

Key words: Haematological, serum, onion powder, treatments.

# Introduction

Feed additives are substances which are added in trace amounts to a diet or feed ingredients to either facilitate feed ingestion, digestion, ingredient dispersion or to preserve the nutritional characteristics of the feed. Additives have been shown to improve fish growth, survival and immunity as well as improve muscle quality (Kim *et al*, 1998, 2000). Other purposes for which additives are in use include, stress resistance, efficiency in feed utilization and elevation of specific immune response systems in fish (Levice *et al.*, 2008; Pandey *et al.*, 2012; Farrag *et. al.*, 2013).

Feed additives are either chemical or botanical in origin. Feed additives of chemical origin include hormones, probiotics (bartocell) and antibiotics (oxytetracycline) and vitamins C and E (El-Banna and Atallah, 2009). These have been criticized for their potential harmful effects on human health and the possible development of resistance by the microbes to these products (Baruah *et al.*, 2008).

Consequently, there is a shift towards the use of additives of plant origin and these include the use of Chinese and Indian herbs, radix (*Angelica simensis*), seeds of *Carica papaya* (Pawpaw) and onion powder (Logambal *et. al.*, 2000; Jian and Wu, 2003; Yin *et al.*, 2009). Onion extract is one of the tested and effective dietary additive that improve the growth of *Clarias gariepinus* (Cho *et al.*, 2010, Cho and Lee, 2012). Onions are very low in calories (about 40 calories/100g) and fats, however rich in soluble dietary fiber. It also contains phytochemical compounds such as allium and allyl disulplide which are converted to allicin by enzymatic reaction when its modified leaves are distorted. Studies have shown that these compounds have anti-mutagenic and anti-diabetic properties. Onions are a rich source of chromium, the trace mineral that helps tissue cells respond appropriately to insulin levels in the blood.

This study was carried out to assess the use of onion powder as an additive in the diet of fingerlings of *C*. *gariepinus*. This particular attempt is aimed at assessing the haematological profile of *C*. *gariepinus* fed for 60 days on diets containing graded levels of onion powder. It is hoped that the outcome of this investigation will provide adequate information on the use of onion powder to facilitate feed ingestion, digestion, and above all build immune stimulation capacity of cultured fish in general and particularly *C. gariepinus*.

## **Materials and Methods**

### Experimental System:

A total of fifteen (15) experimental plastic tanks of 40 liters capacity were used for the trials. The experiment was carried out in the Animal Nutrition Laboratory in Abia State University Umuahia Campus. The tanks were cleaned, disinfected and allowed to dry for 24 hours after which they were filled with dechlorinated water to two-third of the size of the tanks and were covered with a net mesh size 1mm to protect the fish from jumping out of the tanks. Three tanks were used for each experimental diet.

#### **Processing of Onion Powder:**

The raw onions were obtained from the local market in Umuahia, Abia State, Nigeria. The first two dry layers of the raw onions were removed and discarded and the onion bulbs were sliced into smaller sizes using a sharp kitchen knife to

(January-March, 2015)

facilitate drying. The sliced onions were oven dried to a constant weight at  $60^{\circ}$ C. Oven dried onions was blended into a smooth paste in a 3.8 L kitchen-type blender (Warning Products, New Hartford, CT) which was thoroughly cleaned and dried between samples.

## **Proximate Composition Determination:**

Blended onion powder and the experimental diets were analyzed for proximate composition using procedure of AOAC (2000).

# Sourcing and acclimatization of experimental fish:

A total number of three hundred, 3 weeks old *C. gariepinus* fish were collected from a local farm in Umudike, Abia State, Nigeria. They were transported in a 50 liter plastic container to the laboratory and acclimatized for 30 days. During the acclimatization period, fish were fed ad - libitum on a commercial fish feed (Copens feed®).

## Stocking of experimental fish/Experimental Design:

A total of 75 fingerlings were starved for 24hours prior to the commencement of the feeding trial. The fish were randomly sorted, weighed and stocked at the rate of 5 fingerlings per tank. The feeding trial was carried out in fifteen (15) plastic tanks of 60 liters capacity with water depth of 0.40m and the tanks were placed on wooden tables with a height of 1.4m. There were five (5) treatments with fifteen replicates each. The fish were fed to satiation daily at 7:00 hours and 18:00 hours and all fish were subjected to 12:12 light and dark cycle using a natural day and night regime. The experiment was a complete randomized design (CRD).

## Experimental diet:

Each diet as presented in Table 1 was compounded and mixed separately. The mixture was made into pellets using a manual pelleting machine. After pelleting, the feed were sun dried and stored in a sack bag for further use. The fish *C. gariepinus* were stocked five (5) in each plastic tank and they were fed for sixty (60) days on diets containing different levels of onion powder. Fish were fed *ad libitum* daily at 7.00 hours and 18.00 hours. Dead fish were removed and recorded daily.

## Water management:

There was 50% exchange of water in all the tanks daily. Untreated borehole water was used for the study. Water was temporally stored in 50L plastic containers from where it was transferred into the experimental tanks every morning.

## Collection of blood samples from fish:

At the expiration of the experimental period, three fishes from each of the replicates were selected for blood samples. About 2ml of blood were collected into labeled sterile universal bottle containing a drop of ethylene diamineteracetate (EDTA). A second sample of 3ml were collected with a sterile labeled syringe without EDTA and then allowed to coagulate. Blood samples were taken from fish in triplicates on the last day of the experiment. The blood samples that were collected with EDTA were used to determine the hematological indices (Haemaglobin, red blood cell counts, white blood cell counts, and packed cell volume) while the coagulated blood were used for the blood serum (aspertate transaminase, alanine transminase, alkaline phosphate, serum protein, creatinine, blood sugar). The haematological indices of mean cell haemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglobin (MCH) were calculated using the total red blood cell count (RBC), haemoglobin concentration (Hb) and haematocrit (Hct) using the formulae by Sotolu and Faturoti (2011)

MCHC (%) = (Hb/PCV) x 10

MCV (fl) = 
$$PCV/RBC \ge 100$$

In all cases, blood was collected from the caudal peduncle using plastic disposable syringes

## Statistical Analysis:

Data on haematological and serum biochemical parameters were subjected to analysis of variance (ANOVA) using the technique of Obi (1990) and significantly different means were elucidated using Duncan New Multiple range test as outlined by Obi (1990). Differences were considered significant at 5% probability levels ( $p \le 0.05$ ).

# Results

Table 1 shows the composition of the formulated experimental diets. Treatment  $T_1$  contained 0% onion powder and treatments  $T_2$ ,  $T_3$ ,  $T_4$  and  $T_5$  contained 0.5, 1.0, 2.0 and 3.0% onion powder respectively. The result revealed that the diets were generally iso-nitrogenous. The protein content ranged from  $30.8\pm1.86$  to  $32.6\pm2.65\%$  and were not significantly different (p>0.05). Similarly, there were no significant differences (p>0.05) in moisture, fat and carbohydrate content of the experimental diets.

Ingredients (%) Maize	T <sub>1</sub>	$T_2$	т	т	T		
Maize	40.00		$T_3$	$T_4$	$T_5$		
iviai20	49.80	49.80	49.80	49.80	49.80		
Soybean	27.50	27.50	27.50	27.50	27.50		
Onion powder	00.00	0.50	1.00	2.0	3.0		
Wheat Offal	12.00	11.50	11.00	10.0	9.0		
Palm Kernel meal	5.10	5.10	5.10	5.10	5.10		
Fish meal	3.00	3.00	3.00	3.00	3.00		
Bone meal	1.00	1.00	1.00	1.00	1.00		
Vitamin Premix	0.25	0.25	0.25	0.25	0.25		
Methionine	0.10	0.10	0.10	0.10	0.10		
Salt	0.25	0.25	0.25	0.25	0.25		
Palm oil	1.00	1.00	1.00	1.00	1.00		
Total	100	100	100	100	100		
Proximate Composition of diets							
Moisture	1.49±1.11	$1.52 \pm 0.08$	1.45±0.99	$1.50 \pm 0.65$	1.23±0.55		
Crude fiber	0.73±0.23	$0.70\pm0.46$	$0.65 \pm 0.56$	0.64±0.36	0.75±0.89		
Crude protein	30.9±2.33	30.8±1.86	31.2±3.01	32.4±0.99	32.6±2.65		
Ash	4.33±1.14	4.25±0.95	4.30±1.01	5.20±	5.31±0.96		
Fat	4.52±0.99	4.78±0.84	3.96±0.78	4.65±1.05	4.66±1.12		
Carbohydrate	58.03±3.32	57.95±2.08	58.44±1.89	55.61±3.56	55.45±2.10		

(Values are on dry matter basis).

The result in Table 2 shows the haematological responses of fingerlings of *C. gariepinus* fed diets containing various levels of onion powder.

*Haemoglobin* (*Hb*) (%): The haemolgobin content of fingerlings of *C. gariepinus* ranged from 10.57% in fish fed diets containing 0% onion powder to 11.4% in fish fed diets containing 2% onion powder and this value was significantly( $p\leq 0.05$ ) higher.

**Table 2**: Haematological responses of C. gariepinus fingerlings fed diets containing varying levels of onions powder (OP).

Parameters	T <sub>1</sub> 0% (O.P)	T <sub>2</sub> 0.5% (O.P)	T <sub>3</sub> 1% (O.P)	T <sub>4</sub> 2% (O.P)	T <sub>5</sub> 3% (O.P)
Hb (%)	$10.57^{\circ} \pm 0.15$	$11.10^{ab} \pm 0.1$	$10.70^{\rm bc} \pm 0.06$	$11.40^{\circ} \pm 0.1$	$10.90^{\rm bc} \pm 0.1$
PCV (%)	$32.00^{d} \pm 0.46$	$33.30^{b} \pm 0.3$	$31.10^2 \pm 0.17$	$34.20^{a} \pm 0.3$	$32.70^{\circ} \pm 0.3$
WBC ( 10 <sup>6</sup> /ml)	$3.13^{b} \pm 0.01$	$3.07^{d} \pm 0.02$	$3.21^{a} \pm 0.01$	$3.03^{e} \pm 0.01$	$3.11^{\circ} \pm 0.1$
RBC (x10 <sup>6</sup> /ml)	$4.23^{b} \pm 0.01$	$4.11^{d} \pm 0.01$	$4.03^{e} \pm 0.01$	$4.27^{a} \pm 0.01$	$4.14^{\circ} \pm 0.01$
MCV (%)	$1.65^{\rm d}\pm0.01$	$1.70^{\circ} \pm 0.02$	$1.63^{\rm e} \pm 0.01$	$1.70^{b} \pm 0.01$	$1.75^{\mathrm{a}} \pm 0.01$
MCHC (%)	$332.83^{d} \pm 0.64$	$331.27^{e} \pm 1.00$	$340.80^{\circ} \pm 0.69$	$345.37^{a} \pm 0.64$	$341.80^{b} \pm 1.57$

Means and standard deviation (SD) were obtained from triplicate samples. Means in the same row with different superscripts are significantly different ( $p \le 0.05$ ). Hb = Haemoglobin concentration, PCV = Packed Cell Volume, WBC = White Blood Cell, RBC = Red Blood Cell, MCV = Mean Corpuscular Volume, MCH = Mean Corpuscular Haemoglobin, MCHC = Mean Corpuscular Haemoglobin Concentration.

### Packed cell volume (PCV) (%):

The results for the packed cell volume were 32.0% for  $T_1$ , 33.3% for  $T_2$ , 31.1% for  $T_3$ , 34.2% for  $T_4$  32.7% for  $T_5$ . The results showed significant differences (p $\leq$ 0.05) among treatment means. Blood of fishes fed diet containing 2% onion powder had the highest packed cell volume while fishes fed diet containing 1% onion powder had the lowest PCV.

### White blood Cell (WBC) (x10<sup>6</sup>/ml):

The results of the white blood cell count of fingerlings of *C. gariepinus* fed diets containing various levels of onion powder showed significant differences ( $p\leq0.05$ ) in the white blood cell count of the various treatment. The values obtained were  $3.13\pm0.01 \times 10^6$ /ml for T<sub>1</sub>,  $3.07\pm0.02 \times 10^6$ /ml for T<sub>2</sub>,  $3.21\pm0.01 \times 10^6$ /ml for T<sub>3</sub>,  $0.03\pm0.01$  for T<sub>4</sub> and  $3.11\pm0.01 \times 10^6$ /ml for T<sub>5</sub>. This result shows that fishes fed diets containing 1% onion powder had the highest white blood cell count of  $3.21\pm0.01 \times 10^6$ /ml while lowest value of  $3.03\pm0.01 \times 10^6$ /ml was recorded with 2% onion powder.

(January-March, 2015)

The results for the red blood cell counts were  $4.23 \pm 0.01 \times 10^6$ /ml for T<sub>1</sub>,  $4.11 \pm 0.01 \times 10^6$ /ml for T<sub>2</sub>,  $4.03 \pm 0.01 \times 10^6$ /ml for T<sub>3</sub>,  $4.27 \pm 0.01 \times 10^6$ /ml for T<sub>4</sub>, and  $4.14 \pm 0.01 \times 10^6$ /ml for T<sub>5</sub>. There were significant differences (p $\leq 0.05$ ) among the treatment means. Diet containing 2% onion powder had the highest value of red blood cell count of  $4.23 \pm 0.01 \times 10^6$ /ml while diet containing 1% onion powder had the lowest value.

#### Mean Corpuscular Volume (MCV) (%):

The mean corpuscular volume content ranged from  $1.65 \pm 0.01$  T<sub>1</sub> to  $1.75 \pm 0.01$  in T<sub>5</sub>. The treatment means differed significantly (p $\leq 0.05$ ) and was highest in the diet containing 3% onion powder with a value of  $1.75 \pm 0.01$  while the lowest value was recorded in the diet containing 1% onion powder.

### Mean Corpuscular Haemoglobin Concentration (MCHC) (%):

Mean corpuscular haemoglobin concentration in fish fed diets containing various levels of onion powder were  $332.83 \pm 0.64$ . for T<sub>1</sub>,  $331.27 \pm 1.00$  for T<sub>2</sub>,  $340.80 \pm 0.01$  for T<sub>3</sub>,  $345.37 \pm 0.64$  for T<sub>4</sub>, and  $341.8 \pm 1.57$  for T<sub>5</sub> respectively. There were significant differences (p $\leq 0.05$ ) among the treatment means with respect to MCHC values.

#### **Blood serum chemistry:**

The result in Table 3 shows the blood serum chemistry of fingerlings of *Clarias gariepinus* fed diets containing various levels of onion powder.

**Table 3:** Blood serum chemistry of *C. gariepinus* fingerlings fed diets containing varying levels of onions powder (OP).

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Parameters	T <sub>1</sub> 0% (O.P)	T <sub>2</sub> 0.5% (O.P)	T <sub>3</sub> 1% (O.P)	T <sub>4</sub> 2% (O.P)	T <sub>5</sub> 3% (O.P)
TP (mg/dl)	$33.67^{ab} \pm 0.58$	$33.05^{b} \pm 0.03$	$34.07^{a} \pm 0.06$	$32.32^{\circ} \pm 0.02$	$33.79^{a} \pm 0.57$
ALP (U/C)	$24.82^{ab} \pm 0.12$	$23.90^d\pm0.26$	$25.03^{a} \pm 0.06$	$24.20^{\circ} \pm 0.1$	$24.63^{b} \pm 0.01$
Urea (mg/dl)	$1.31^{ab} \pm 0.01$	$1.24^{d} \pm 0.03$	$1.29^{\rm bc} \pm 0.01$	$1.33^{a} \pm 0.01$	$1.27^{cd} \pm 0.01$
Creatinine (mg/dl)	$0.41^{d} \pm 0.01$	$0.44^{\mathrm{a}} \pm 0.01$	$0.42^{\circ} \pm 0.01$	$0.40^{\circ} \pm 0.01$	$0.43^{\text{b}} \pm 0.01$
Glucose (mg/dl)	$2.28^{ab} \pm 0.81$	$1.60^{b} \pm 0.56$	$1.60^{b} \pm 0.56$	$1.85^{a} \pm 0.61$	$0.413^{\circ} \pm 0.72$

Means and standard deviation (SD) were obtained from triplicate samples. Means in the same row with different superscripts are significantly different ( $p\leq0.05$ ). ALP = Alkaline Phosphate, TP = Total protein, TCHO = Total Carbohydrates.

#### Total protein (TP) (mg/dl):

Total protein concentration in blood of experimental fish fed diets containing 0%, 0.50%, 1%, 2% and 3% were 33.67 mg/dl, 33.05 mg/dl, 34.07mg/dl, 32.32 mg/dl and 33.79 mg/dl respectively. There were significant differences among the treatment means. Highest value of serum total protein was recorded in the fish fed 1% onion powder and lowest in the diet containing 2% onion powder.

## Alkaline Phosphate (ALP) (iu/l):

Blood serum of *C. gariepinus* fed experimental diets containing 0%, 0.50%, 1%, 2% and 3% had the following ALP concentrations,  $24.82 \pm 0.12$ ,  $23.90 \pm 0.26$ ,  $25.03 \pm 0.06$ ,  $24.20 \pm 0.1$  and  $24.63 \pm 001$  respectively. There were significant differences (p≤0.05) among the treatment means.

### Urea (mg/dl):

The results for urea in blood of fishes fed varying levels of onion powder were  $1.31 \pm 0.01$  for  $T_1$ ,  $1.24 \pm 0.03$  for  $T_2$ ,  $1.29 \pm 0.01$  for  $T_3$ ,  $1.33 \pm 0.01$  for  $T_4$  and  $1.27 \pm 0.01$  for  $T_5$  respectively. There were significant differences (p $\leq 0.05$ ) among the treatment means.

### Creatinine (mg/dl):

The creatinine values ranged from 0.41  $\pm$  0.01 in T<sub>1</sub> to 0.44  $\pm$  0.01 in T<sub>2</sub>. The values obtained werse 0.41  $\pm$  0.01 for T<sub>1</sub>, 0.44  $\pm$  0.01 for T<sub>2</sub>, 0.42  $\pm$  0.01 for T<sub>3</sub>, 0.40  $\pm$  0.01 for T<sub>4</sub> and 0.43  $\pm$  0.01 for T<sub>5</sub>. There were significant differences (p≤0.05) among the treatments.

#### Glucose (mg/dl):

Glucose concentration in the serum of *C. gariepinus* fingerlings were  $2.28 \pm 0.8$  mg/dl for T<sub>1</sub>,  $1.60 \pm 0.56$  mg/dl for T<sub>2</sub>,  $1.60 \pm 0.56$  mg/dl for T<sub>3</sub>,  $1.85 \pm 0.61$  for T<sub>4</sub> and  $0.413 \pm 0.72$  mg/dl for T<sub>5</sub> respectively and also indicate significant differences in treatment means.

# Discussion

Haematological parameters are valuable component in monitoring feed toxicity especially with feed constituents that affect the formation of blood (Oyawoye and Ogunkunle, 1998). The haematological parameters of juvenile *C. gariepinus* fed diets containing graded levels of onion powder showed significant differences among the treatments in Hb, PCV, WBC, RBC, MCV and MCHC. Other researchers (Bello et al., 2014, Martins et. al 2002, Azza *et. al.* 2009) reported that onion bulb (*Allium cepa*), *A. sativum* and walnut leaf extract residues, in fish feed similarly induced increase in blood parameters.

#### (January-March, 2015)

The haemoglobin (Hb) values in this study indicate that the fish were not anaemic. This is because Hb levels recorded in this study were comparable with literature on RBC and Hb in *C. gariepinus* (Adeyemo *et al.* 2003; Adewoye, 2010; Anyanwu *et al.* 2011; Ozovehe, 2013, Anene *et al.* 2014). Haemoglobin component is crucial to the survival of fish, and is directly related to the oxygen binding capacity of blood (Tatina *et al.*, 2010). The high haemoglobin concentration obtained in the fish fed diet containing 2% onion powder could be an indication of high oxygen absorption and transportation capacity of the cells of the experimental fish.

The white blood cell (WBC) of fish is important in the disease fighting capacity of experimental fish. Low WBC is a decrease in the number of disease fighting cells circulating in the blood of fish. The results of this study show that there is an increase in WBC with increase in the level of onion powder in the diet. This suggests that the onion powder enhanced the capacity of the experimental fish to build immunity against pathogens. The findings of this study were in tandem with Bello *et al.*, (2014) who reported that WBC increases in fish fed diets supplemented with onion bulb and walnut (*Tetracarpidium conophorum*) leaf. Gazuwa *et al.*, (2013) and Bello *et. al.* (2014) reported that raw and cooked. *A. cepa* contain phytochemicals that include alkaloids, flavonoids, terpenes, steroids, glycosides, tannin and thiosulfinates. These compounds may play a role in the immune stimulation and in the function of organs (spleen, thymus and bone marrow) that is related to blood cell formation (Bello *et. al.* 2014). It is also possible that the increase in WBC may result from the stimulation of lymphomyeloid tissues as a mechanism of the experimental fish to tolerate the presence of sub-lethal concentrations of the above mentioned alkaloids known to be constituents of *Allium cepa* (Ates *et al.*, 2008; Cho *et al.*, 2012).

The PCV of *C. gariepinus*\_obtained in this study were within the range of the corresponding values described by (Akintayo *et. al*, 2008, Ozovehe, 2013). A reduced concentration of PCV is always an indication of the presence of substances such as haemoglutinin (Bello *et. al.* 2014) which is capable of reducing the blood forming ability of the fish. Increasing PCV levels as was recorded in this study indicate the absence of such toxins in the blood of the fish.

There were significant differences ( $p \le 0.05$ ) in the activities of serum enzyme. The transaminase (Alkaline phosphates) is an important enzyme for monitoring the health status of fish (Racicot et al, 1975). The values of ALP obtained in this study were relatively low when compared with literature (Ozovehe, 2013, Anene *et. al*, 2014), decrease in ALP could be as a result of liver damage, leakage of enzymes from the liver into the serum, excessive fat digestion and possibly a low nitrogen diet (Anene *et al*, 2014).

The total protein value in *C. gariepinus* as reported in (Akintayo et al, 2008, Agbabiaka *et al*, 2013) was higher than values of 33.05 - 34.07 mg/dl obtained in this study. Environmental conditions of rearing facilities and handling have been shown to influence blood protein levels and may be attributed to the differences in total blood protein records (Ayoola, 2011) which is applicable in this study.

The blood sugar levels in *C. gariepinus* in this study ranged from  $0.413 \pm 0.72$  to  $2.28 \pm 0.81$  mg/dl and were significantly lower than literature reports (Shakoori *et al.*, 1996; Tavares-Dias, 2000; Yilmaz *et al.*, 2006, Akintayo, 2008, Anene *et. al.* 2014). These differences in blood sugar levels may be due to differences in the chemical composition of the diets. This study has shown that increases in the level of onion powder in the experimental diet lead to significant reduction in the blood glucose level of juvenile and may indicate the obvious effect of onion powder on blood sugar levels in juvenile *C. gariepinus*.

### Conclusion

The results of this study showed that inclusion of onion powder in the experimental diets of *C. gariepinus* significantly affected the haematological indices here studied. Most specifically juvenile *C gariepinus* that received diets with onion powder had relatively higher Hb, RBC, WBC values which indicate that onion powder could increase the immune response capability of the fish. The results also show that blood glucose levels in fingerlings of *C. gariepinus* can be reduced with the inclusion of onion powder in their diets. Significant differences were similarly recorded in the values of ALP, urea and creatinin in fingerlings of *C. gariepinus* fed diets containing various levels of onion powder. Most of the parameters here studied peaked at 2% level of inclusion of onion powder.

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