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Biochemical Profile of *Clarias gariepinus* (Burchell, 1822) Juveniles Fed Blood Meal-Bovine Rumen Digesta (BMBRD) Included Diets

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Abstract

The effect of BMBRD diets fed *Clarias gariepinus* (Burchell, 1822) juveniles was investigated on the liver enzymes and creatinine metabolite with a view to revealing underlying physiological conditions of the organ of the fish. Three iso-nitrogenous experimental diets containing 35% crude protein were formulated with 25% and 50% BMBRD inclusion level to substitute the fishmeal component which was the primary protein source while the 0% BMBRD served as the control. Sixty (60) *C. gariepinus* was obtained and stocked in glass aquaria (60cm x 30cm x 30cm) at a stocking density of 10 fish per tank and were fed 4% of the body weight in two installments for 10 weeks. After the tenth week of experimental feeding, five fish specimens were selected from each tank and sacrificed for processing of the selected biochemical assays. Analyses of the results showed that *C. gariepinus* juveniles fed 25% BMBRD diet had significantly higher level ($p<0.05$) of ALT, ALP and LDH activities in their liver than in the liver of the fish juveniles fed 0 and 50% BMBRD included diets. The level of creatinine was however significantly higher ($p<0.05$) in the liver of the fish fed the control and 25% BMBRD diet. The study concluded that BMBRD has no adverse effect on the liver enzymes and metabolite of *C. gariepinus*.

Keywords

Clarias gariepinus, Blood Meal, Rumen Digesta, Diets, Biochemical Enzymes

1. Introduction

Biochemical assays are analytical *in vitro* procedures used to detect or measure the binding or activity of a biological molecule such as an enzyme [1]. Assays are vital for the study of enzyme kinetics and enzyme inhibition [2]. According to Singh *et al.* [3] and Aladesanmi *et al.* [4] biochemicals are the assessable body contents for checking the toxicity of any chemicals and the results of such biochemical parameters may result in serious outcome in the form of various diseases in fishes or animals. Biochemical parameters also reveal underlying physiological conditions of the organs or tissues of organisms [5]. Alanine Aminotransferase (ALT) is an enzyme found primarily on the liver and kidney originally referred to as 'serum glutamic pyruvic transaminase' [5]. ALT which is an enzyme produced by liver cells helps the body metabolise proteins [6]. A low level of ALT has been used to screen for and/ or monitor liver disease. ALT is usually measured concurrently with Aspartate Aminotransferase (AST) as part of a liver function panel to determine the source of organ damage. Various studies in fish research have used the determination of ALT and AST activities to indicate bacteria, viral and parasitic infections, intoxications and water pollution [7-9].

Alkaline phosphatase (ALP) is a hydrolase enzyme responsible for removing phosphate groups from many types of molecules, including nucleotides, proteins and alkaloids

[10]. ALP is involved in bone growth and excreted in the bile. It may be elevated if bile excretion is inhibited by liver damage [11]. A change in ALP activity reflects a change in endoplasmic reticulum mass and also attributed an increase in ALP activity in the liver of fish to cellular damage [12]. A lactate dehydrogenase (LDH or LD) is an enzyme found in nearly all living cells (animals, plants, and prokaryotes) [13] which generally associated with cellular metabolic activities [9] and such activities are inhibited under stress especially after exposure to toxic substances [14]. The objective of this work is therefore to investigate the effect of Blood meal-bovine rumen digesta blend diets on the liver enzymes and creatinine metabolite of *C. gariepinus* juveniles.

2. Materials and Methods

Preparation of Blood Meal-Bovine Rumen Digesta (BMBRD)

The bovine blood and rumen digesta were collected freshly and separately into two different clean plastic buckets from slaughter slab. The bovine blood which was thoroughly stirred with a paddle to prevent coagulation was mixed with rumen digesta obtained from eviscerated cattle in the ratio 1:1 (weight/weight) [15]. The mixture was heated for 50 minutes with constant stirring until it is almost free of moisture content and was sun dried for five days on a big clean dry polythene sheet. The dried BMBRD was then blend and packed in an airtight container until further needed.

2.1. Diet Formulation and Preparation

Three approximately isonitrogenous 35% crude protein experimental diets were formulated using fish meal, BMBRD, yellow maize, wheat offal, mineral and vitamin premix [15] based on their proximate composition using Pearson's square method [16]. Fish meal component which is the main source of protein was substituted with the BMBRD at 25% and 50% inclusion levels while the formulated diet without BMBRD inclusion served as the control diet. The prepared diets were then sun-dried for 3 days to prevent deterioration. Each of the prepared diets was labelled 0%, 25%, and 50% BMBRD respectively in plastic containers and stored in a cool environment until needed for the feeding trials.

2.2. Experimental Diet Feeding

Sixty (60) *Clarias gariepinus* juveniles (Av. Wt. 36.95 ± 1.60g) obtained from a reputable Fish Farm in Ile-Ife, Nigeria were randomly distributed into six labelled glass aquaria (60 cm x 30 cm x 30 cm) at the rate of 10 fish per tank and were left to acclimatize for period of two (2) weeks in Fish Culture Laboratory, Department of Zoology, Obafemi Awolowo University. During the period of acclimatization, the fish were fed on Durante feed (2mm) at the rate of 4% of their body weight per day in two installments to ensure that the experimental fish had a uniform nutritional status before being exposed to the experimental diets. After two (2) weeks of acclimatization, the fish which were stocked in duplicates were fed the experimental diets at 4% of their body weight in two installments between 8:00-9:00 am and 5:00-6:00 pm for a period of 10 weeks [15].

2.3. Biochemical Evaluation of the Experimental Diets

After the tenth week of experimental feeding, five fish from each of the treatments were selected and sacrificed for biochemical assays. The liver of the fish specimens were carefully dissected and placed in an ice tray. Each of the liver was weighed and homogenized in ice-cold 10 mL tris buffer (pH 7.8) using a Trison homogenizer. The homogenates were centrifuged at 2000 rpm for 10 minutes. The supernatant was then carefully decanted into a labelled Teflon tubes, kept frozen (-4°C) overnight to ensure maximum release of the enzymes from the liver cells.

2.3.1. Determination of Alkaline Phosphatase (ALP)

A Randox kits was used for determination of ALP following the method described by Bassey *et al.* [17] as modified by Wright *et al.* [18]. Into a cuvette, 10 µl of sample was mixed:^#with 500 µl of the Randox reagent. The initial Absorbance was read at 405 nm wavelength, and subsequently every minute for over 30 minutes. The mean Absorbance per minute was then used for ALP activity calculation:

ALP activity (U/I) = 2760 x ΛA 405 nm/min;

Where 2760 = Extinction coefficient

ΛA 405 nm/min = Change in absorbance per minute for the homogenate sample

2.3.2. Assay for Alanine Transaminase (ALT) Activity

The method described by IFCC [19] using Randox kits was used for the determination ALT activities. Fifty (50) µl of the sample and 500 µl of the ALT reagent were mixed in a test tube, and the initial Absorbance at 546 nm wavelengths was read after 1st minute. The timer was started simultaneously and further readings of the Absorbance were taken after 1st, 2nd, and 3rd minutes. The ALT activity was then calculated as:

$$\text{ALT activity (nm/min)} = 1746 \times \Lambda A\ 546\ \text{nm/min:}$$

Where 1746 = Extinction coefficient

ΛA 546 nm/min = Change in absorbance per minute for homogenate sample

2.3.3. Aspartate Transaminase (AST) Activity Determination

The assay method described for ALT was used with the exception that the ALT reagent was replaced with the AST reagent [20]. AST activity was then measured as:

$$\text{AST activity (nm/min)} = 1746 \times \Lambda A\ 546;$$

Where 1746 = Extinction Coefficient

ΛA 546 nm/min = Change in absorbance per minute for the homogenate sample.

Assay for Lactate Dehydrogenase (LDH) Activity)

The method described by Reitman and Frankel [21] was used for LDH using a Randox kits for the determination. Into a test tube, 0.01 ml of sample was mixed with 0.50 ml of the Randox reagent. The initial Absorbance at 546 nm was read after 0.5 minute. The timer was started simultaneously and further readings of the Absorbance were taken after 1, 2, and 3 minutes. The LDH activity was then calculated as:

$$\text{LDH activity (nm/min)} = 7647 \times \Lambda A\ 365;$$

Where 1746 = Extinction Coefficient

ΛA 365 nm/min = Change in absorbance per minute for the homogenate sample.

2.3.4. Creatinine Determination

The method described by Aitken *et al.* [20] was used for creatinine level using a Randox kits for the determination. Two test-tubes labelled S for standard and T for sample, and 1 ml of the Randox reagent was put into the test-tubes followed by the introduction of 0.1 ml of the standard solution into S (test-tube) and 0.1 ml sample into T (test-tube). The content of each test-tube was gently mixed, distilled water was used to zero the automatic chemical analyzer and after 30 seconds, the initial Absorbance (A_1) of the standard and sample was read and exactly 2 minutes later, final Absorbance (A_2) was read for both the standard and sample. Distilled water was used for a blank test and the concentration of creatinine was then calculated as:

$$A_2\text{-}A_1 = \Lambda A_{\text{sample}} \text{ or } \Lambda A_{\text{standard}}$$

$$\frac{\Lambda A_{\text{sample}}}{\Lambda A_{\text{standard}}} = \text{x Standard conc. (mg/dl)} = \text{mg/dl}$$

3. Results

The Alanine Aminotransferase activity in liver of *Clarias gariepinus* fed the experimental diets and the control is shown in Figure 1. The result showed that *C. gariepinus* juveniles fed 25% BMBRD diet had significantly higher level ($p<0.05$) of ALT activity than in the fishes fed 0% and 50% BMBRD diets. The lowest ALT activity was however recorded in the liver of fish fed 50% BMBRD diet.

Figure 2 shows the Aspartate Aminotransfarase activity level in the liver of *C. gariepinus* juveniles fed the experimental diet. The AST level in the liver of fish fed with control (0%BMBRD) diet was found to be significantly higher ($p<0.05$) than in the fishes fed the experimental BMBRD included diets. However, the level of AST activity in liver of fish fed the BMBRD included experimental diets were not significantly different ($p>0.05$) from each other.

As shown in Figure 3, the Alkaline Phosphatase activity level in liver of *Clarias gariepinus* juveniles fed with 25% BMBRD diet was significantly higher ($p<0.05$) than the ALP activity level in liver of the fish fed the 0% and 50% BMBRD diets. The result also revealed that ALP activity level in liver of fish fed 50% BMBRD diet was higher (though not significantly, $p>0.05$) than the ALP activity level of the fish fed the control diet (0% BMBRD).

The activity of Lactate dehydrogenase in liver of *C. gariepinus* fed different experimental and control diets is shown in Figure 4. Although, the LDH activity in liver of *C. gariepinus* fed with 25% BMBRD diet was higher than in the liver of fish fed 0% BMBRD diet (control diet), the differences in the activity levels were found not to be significantly different ($p>0.05$) from each other but were significantly higher ($p<0.05$) than the activity of the enzyme in the fish fed 50% BMBRD diet.

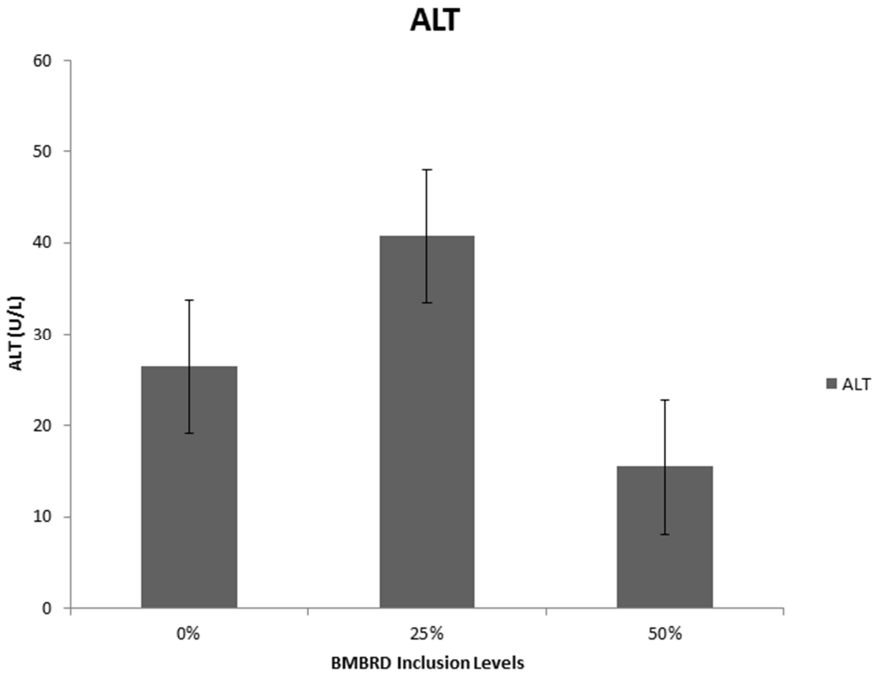

Figure 1. *Alanine aminotransferase activities in liver of C. gariepinus juveniles fed experimental diets.*

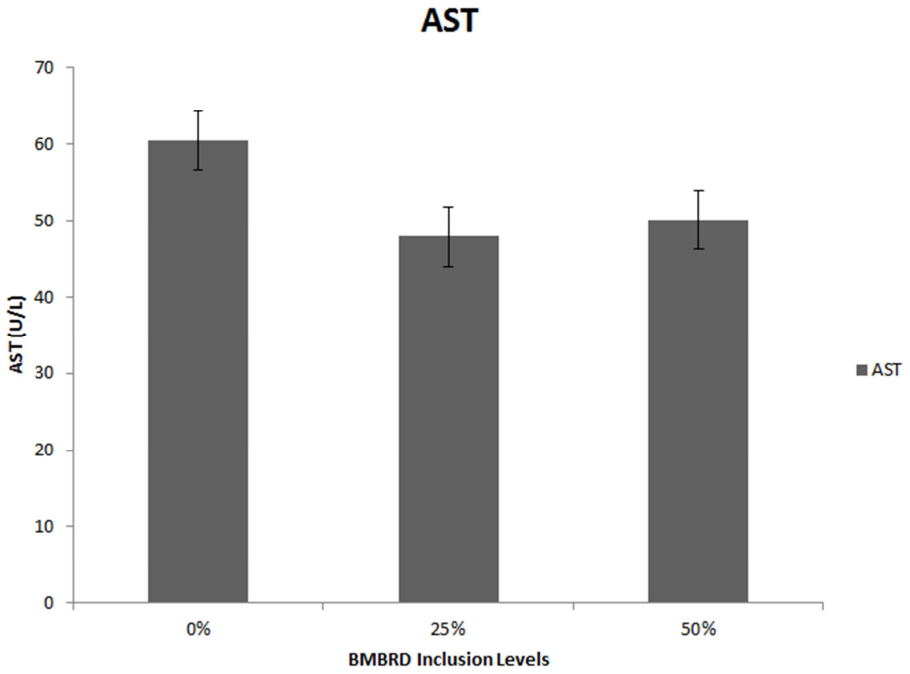

Figure 2. *Aspartate aminotransfarase activities in liver of C. gariepinus juveniles fed the experimental diets.*

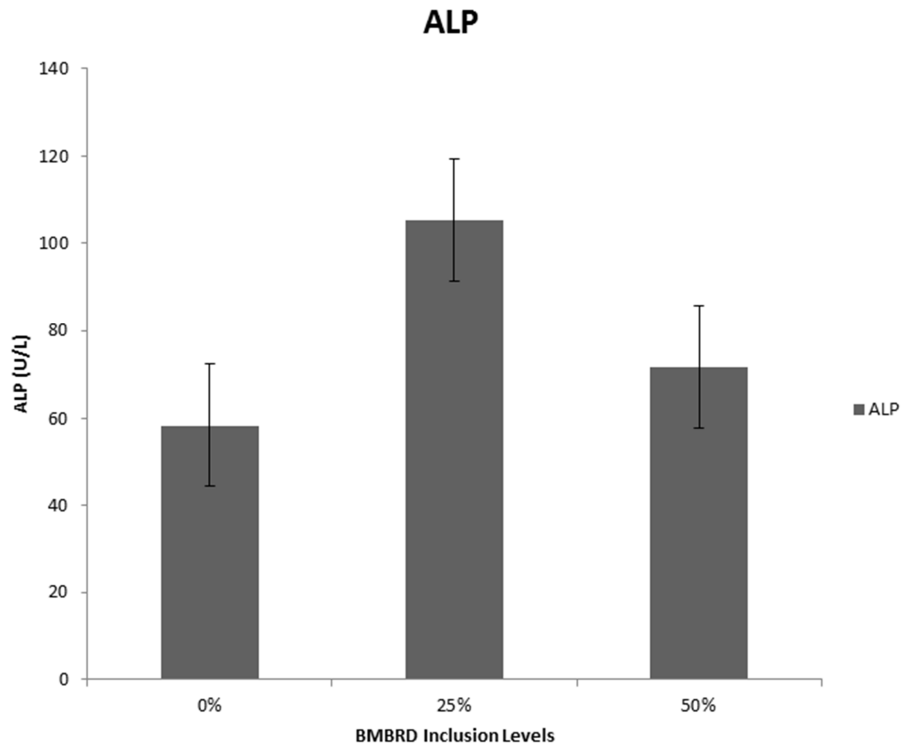

Figure 3. *Alkaline phosphatase activities in liver of C. gariepinus juveniles fed the experimental diets.*

The activities of the metabolite in the liver of *Clarias gariepinus* juveniles fed the experimental and control diets were shown to be significantly higher ($p<0.05$) in the fish fed 0% and 25% BMBRD diets compared with those of fish fed 50% BMBRD diet (Figure 5). However, comparative analyses of the level of the creatinine metabolite in the fish fed 0% and 25% BMBRD diets showed no significant differences ($p>0.05$) in the activity level of the metabolite.

4. Discussion

Biochemical is the assessable body contents for checking the toxicity of any [3]. In this study, the observed alanine transaminase (ALT) activity in the liver of fish fed 25% BMBRD diet was higher (40.70 U/L) than in the liver of fish fed 0% BMBRD diet (26.48 U/L) while the liver ALT

activity in fish fed 50% BMBRD was lower (15.46 U/L) than in the liver of fish fed no BMBRD (control). A similar result was reported by Gabriel *et al.* [23] who reported that ALT activity in the liver of *C. gariepinus* exposed to cypermethrin (an insecticide) ranged from (18.35 to 50.0 U/L) with the activity of enzyme increasing with the concentration of the toxicant. Dienye and Olumuji [22] reported a lower level of ALT activity in the serum of *C. gariepinus* fed different levels of Moringa leaf diet with values ranging from (11.40 to 12.80 UL^{-1}). The increase in liver ALT activity of fish fed 25% BMBRD in this study when compared with those of the control probably indicated cellular malfunction in the fish [24] and the decreased level in the liver of the fish fed 50% BMBRD diet could probably be associated with suggested disruption of the transfer of the α-amino acid group of alanine to apha-ketoglutarate which results in the formation of pyruvic acid [23]. An elevated value in the fish fed 25% BMBRD included diet could probably be associated with some form of liver disease [5]. ALT and AST activities were reported to be biological responses of severe hepatic injury in fish and the bioassays have been used as a diagnostic tool for estimating necrosis of liver cells [25, 9].

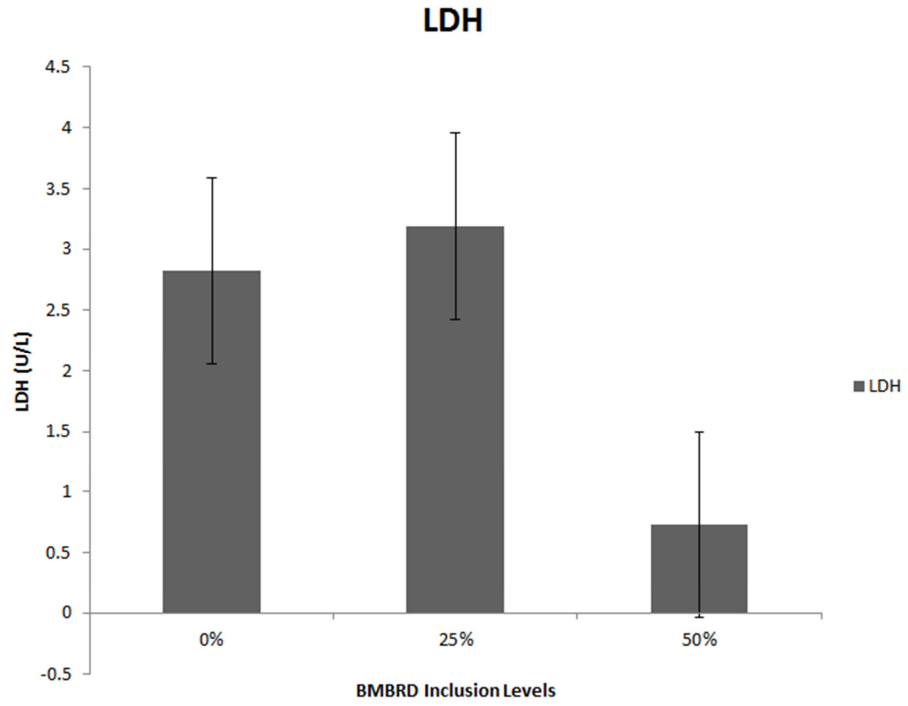

Figure 4. *Lactate dehydrogenase activities in liver of C. gariepinus juveniles fed the experimental diets.*

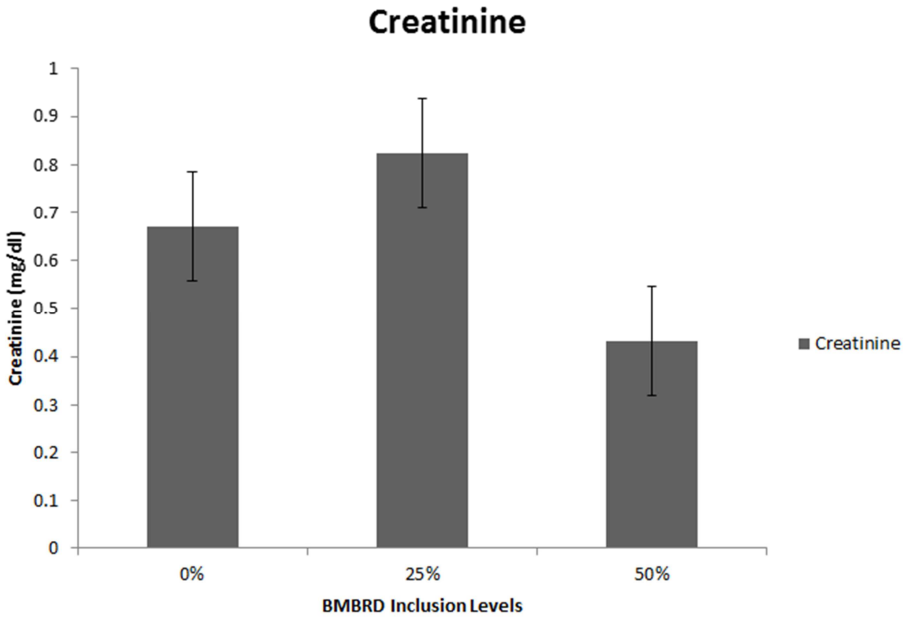

Figure 5. *Creatinine level in the liver of C. gariepinus fed the experimental diets.*

The liver aspartate transaminase (AST) activity of fish fed 0% BMBRD (60.42 U/L) was higher than in the liver of fish fed 25% BMBRD diet (47.88 U/L) and 50% BMBRD diet (50.06 U/L). Gabriel *et al.* [23] reported a very high value of between 31.65 to 123.0 U/L for AST activity in liver of *C. gariepinus* exposed to cypermethrin concentration. However, [22] also reported AST activity in serum of *C. gariepinus* fed different levels of moringa leaf to range from (20.20-22.80 UL^{-1}). The decrease in the AST activity in this study when compared with other studies probably indicated that active transamination and oxidative deamination took place in the fish fed the experimental and the control diet [23].

Alkaline phosphatase (ALP), a hydrolase enzyme responsible for removing phosphate groups from organic molecules [26], was found to have relatively high activity in catfish juveniles fed the experimental and the control diets during the period of study. The ALP activity was higher in the liver of fish fed 25% BMBRD diet (105.23 U/L) than in the liver of fish fed the control diet (58.33 U/L) and 50% BMBRD diet (71.61 U/L). Edori *et al*. [27] reported an increased level of ALP activity in liver of *C. gariepinus* exposed to Paraquat (an herbicide), where the ALP values obtained for the fish ranged between 51.63-262.50 U/L respectively, a value which was found to increase as the concentration of Paraquat increases. [22] however reported a value ranging between 46.80 to 58.40 UL^{-1} in the serum of *C. gariepinus* fed different inclusion level of Moringa leaf diet. The increased ALP activity in the liver of fish fed 25% BMBRD and 50% BMBRD diet, when compared with the control in this study probably indicated the possibility of membrane damage because ALP is a membrane bound enzyme [10]. Under stressful condition, the enzyme activity is affected [28]. A change in ALP activity reflects a change in endo-reticulum mass [11]. An elevated level of the enzyme in liver of the fish could probably be related to cellular damage [28] probably due to some stressful conditions which were diet related.

Lactate dehyrogenase (LDH) activity obtained in this study dropped in liver of fish fed 50% BMBRD diet compared with the LDH activity obtained in the liver of fish fed the control diet. Similar result was reported by [29] who reported decreased activity of LDH in *Cyprinus carpio* when exposed to Cypermethrin in time periods of 48 and 96 hrs. The decreased in LDH activity probably indicated a decrease in the glycolytic process due to the lower metabolic rate [22]. Activity of the enzyme has been reported to be inhibited under stressful condition [14].

The creatinine level observed in the liver of fish fed 25% BMBRD diet (0.82 mg/dl) was higher than in the liver of fish fed 0% BMBRD diet (0.67 mg/dl) and 50% BMBRD diet (0.43 mg/dl). [30] reported the creatinine levels in the liver of *C. gariepinus* in three different commercial ponds ranged between 0.13 to 1.30 mg/dl. Although the values were still within the range reported by these authors, however, the elevated level of this metabolite could be attributed to the stress the fish were probably exposed to during the period of handling prior the termination of the experiment.

5. Conclusion

The present study revealed that the BMBRD diets were accepted and digested by the experimental fish without any adverse effect on the liver enzymes and metabolite of *C. gariepinus,* although there were variations in the values obtained which could be attributed to the stress the fish passed through during cultured period and not due to the BMBRD inclusion in the diets. The study however confirmed that BMBRD diet up to 25% could replace fish meal in commercial diet of *C. gariepinus* with better impact on the liver enzymes and metabolite of *C. gariepinus*.

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