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Effects of Betaine and B-Glucan Co-Supplementation on Serum Biochemistry of African Catfish (Clarias Gariepinus) Fingerlings

*[1,2]Ali, M. E., [2]Sogbesan, O. A., [3]Abubakar, K. A. and [4]Danba, E. P.

[1]National Biotechnology Research and Development Agency, Billiri, Gombe State, Nigeria
[2]Department of Fisheries, Modibbo Adama University, Yola, Adamawa State, Nigeria
[3]Department of Zoology, Modibbo Adama University, Yola, Adamawa State, Nigeria
[4]Department of Biological Sciences, Taraba State University, PMB 1167 Jalingo, Taraba State, Nigeria
*Correspondence author: mealikumbo@gmail.com

Abstract

Feed suitability is one of the factors that strongly influence the serum biochemistry of fish. The effects of betaine and β-glucan co-supplementation on serum biochemistry of African catfish (Clarias gariepinus) fingerlings were evaluated. Betaine and β - glucan were co - supplemented at different dose into formulated feed with 40% crude protein at; 0.0g/100g (BBG0 i.e. control), 0.325g/100g (BBG1), 0.75g/100g (BBG2), 1.125g/100g (BBG3) and 1.50g/100g (BBG4)in triplicate, fed to C. gariepinus fingerlings(n = 300, 10.0±0.00g) in fifteen (15) plastic tanks (n = 20) at a fixed feeding rate of 3% body weight twice daily for twelve (12) weeks. Blood samples were collected for serum biochemical analysis. The water quality parameters were within the recommended ranged for C. gariepinus. Serum values of Total protein (4.20±0.4- 4.75±0.3 g/dL), albumin (2.60±0.1- 2.75±0.1 g/dL), globulin (1.60±0.07-2.00±0.02g/dL), albumin/globulin (A: G) ratio(1.38±0.3 - 1.63±0.4), aspartate aminotransferase i.e. AST(32.00±1.9- 32.90±3.7 U/L),alanine aminotransferase i.e. ALT (53.70±2.1- 55.00±3.5 U/L), glucose (304.00±22-320.00±11 mmol/L), potassium (2.98±0.2-3.74±0.3 mmol/L), sodium (136.30±11.5-140.10±11.4 mmol/L) and chlorine (107.30±10.2 - 110.70±6.7 mmol/L) respectively were recorded. These changes observed in the serum biochemistry of C. gariepinus fingerlings indicated that betaine and β-glucan co-supplementation at different doses employed in this research did not result in statistically significant (p > 0.05) changes, but had a positive effect on physiology by increasing the total serum protein and lowering the serum carbohydrate values as compared to the control feed.

KEYWORDS: Changes, Co-Supplementation, Fingerlings, Formulated Feed, Physiology.

INTRODUCTION

In aquaculture, studies on the haematological and serum biochemical parameters of cultured fish are crucial for monitoring their health during cultivation (Abdel-Hay *et al.*, 2021). Haematological and serum biochemical parameters indicate the suitability of feed, physiology, and environment of cultured fish (Rawung *et al.*, 2019). Responses to feed, pollution, and stress, as well as ecological and physiological factors, affect the haematological and serum biochemical parameters of fish (Okey *et al.*, 2022). Changes in serum biochemical parameters depend on fish species, age, sexual maturity cycle, and health condition (Rawung *et al.*, 2019). The key variations that occur in serum biochemistry related to feed are fluctuations in protein, glucose, cholesterol, and other serum biochemical components (Ndatsu *et al.*, 2022). Haematological and serum biochemical studies are particularly useful in assessing a fish's physiological and pathophysiological status since morphological and biometric parameters alone do not always provide a complete picture (Abdel-Hay *et al.*, 2021). However, reliable reference ranges for comparison are necessary for the data on haematological and serum biochemical parameters of fish species to be meaningful (Ndatsu *et al.*, 2022). There is still a scarcity of published literature establishing normal haematological and serum biochemical values and reference ranges for numerous fish species.

Normal values exist for only a handful of haematological and serum biochemical parameters, and the established values tend to have a wide range due to a lack of standardisation between methods (Abdel-Hay *et al.*, 2021). Research on supplementation or co-supplementation of immune-nutrients into fish feed may have effects on fish serum biochemistry.

Betaine is one of these immune nutrients that promotes growth and increases serum protein contents in fish (Jamil *et al.*, 2019; Abed Ali and Al-Faragi, 2017). Betaine, also called trimethyl glycine (TMG), glycine betaine, lycine or ox neurine, is the trimethyl derivative of the amino acid glycine, a naturally occurring compound found in numerous plants and animal tissues (Abed Ali and Al-Faragi, 2017). Due to its osmotic properties, betaine has the potential to improve the digestibility of specific nutrients. It is involved in protein and energy metabolism due to its methyl group donor function (Murthy *et al.*, 2016).

ß-glucans are another immune nutrient. ß-glucans are the most popular immune-nutrient in aquaculture for improving growth and stimulating serum biochemistry profiles in fish (Huong et al., 2016; Abed Ali and Al-Faragi, 2017; Sánchez-Martínez *et al.*, 2017). ß-glucans are polysaccharides of D-glucose monomers, which form the structure of many cell walls of yeast, fungi, algae, and cereal grains. The glucose units are joined together by glycosidic bonds in various conformations, resulting in either a branched or linear structure (Arena *et al.*, 2017). ß-glucans have different primary molecular structures that vary depending on their source, which have different implications on their immune-nutrient activity (Vetvicka and Vetvickova, 2016). Sánchez – Martínez *et al.* (2017) reported that findings have shown that supplementation of fish feed with ß-glucans (1,3/1,6), which are extracted from yeast (*Saccharomyces cerevisiae*), resulted in changes in haematological and serum biochemical parameters in both salt and freshwater fish, such as Atlantic salmon (*Salmo salar*), rose snapper (*Lutjanus guttatus*), sea bass (*Dicentrarchus labrax*), grass carp *Ctenopharyn godon Idella*), rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*).

Clarias gariepinus is the most cultured and economically important freshwater fish species that contributes immensely to the annual freshwater fish production in Nigeria, it is readily acceptable among Nigerian fish farmers and consumers; hence, it commands high commercial values, and is an important source of animal protein, it also serves as a model organism in research, it is cultured intensively and extensively in Africa, Europe and Asia. The economic benefits of this fish species are due to its hardiness, fast growth, large size attainable, and ability to withstand and tolerate harsh environmental conditions (Awodiran *et al.*, 2019).

Research indicated that betaine, when added to fish feed, promotes growth in common carp (*Cyprinus carpio*) fingerlings and also that ß-glucans supplementation into fish feed increases the serum protein contents and decreases the serum carbohydrate contents in striped catfish (*Pangasianodon hypophthalmus*) fingerlings (Huong *et al.*, 2016; Abed Ali and Al-Farag, 2017). In light of the above information, there is little or no research to determine the efficiency of betaine and ß-glucan co-supplementation on serum biochemistry of African catfish *C. gariepinus*. This research contributes important data on the normal serum biochemical parameters of cultured *C. gariepinus*, for which little data exist. The data from this research are part of the study by Ali (2022) on the performance and cost-benefit of African mud catfish (*C. gariepinus*) fed different levels of dietary betaine, ß-glucan, and co-mixed additives diets. Therefore, this research was designed to evaluate the effects of betaine and ß-glucan co-supplementation on serum biochemistry of African catfish (*C. gariepinus*) fingerlings.

MATERIALS AND METHODS

Study Area

The research was conducted at Lay-Joy Fish Farm, Gombe-Yola road, Billiri local government area (LGA), Gombe State, Nigeria. Billiri LGA, which lies within Latitude 9°50'N; 11°09´E and Longitude 9.833°N 11.150°E, covers an area of 737km² (285 sq. m) and is 50 km away from Gombe, the State capital.

Experimental Fish

Three hundred (300) *C. gariepinus* fingerlings with a mean initial weight (10.0±0.00g) were stocked at twenty (20) fingerlings per tank in triplicate per treatment after one (1) week of acclimatisation. The study lasted for twelve (12) weeks.

Experimental Feed

The formulated feed contained: fish meal (FM), soybean meal (SBM), yellow maize meal (YMM), groundnut cake meal (GNCM) with betaine and ß-glucan co-supplements. All ingredients were ground into a fine powder using a hammer mill and sieved through a 0.25 mm sieve. Fish meal, soybean meal, groundnut cake meal and yellow maize meal were obtained from commercial suppliers in Gombe, the vitamin/mineral premix, fish oil and chromic oxide (Cr_2O_3) were

purchased from TTS Integrated Farms Lagos, while the betaine powder naturally derived from sugar beets (*Beta vulgaris*) and the ß -glucan (ß - 1,3/1,6 - D - glucan) powder naturally derived from baker's yeast (*S. cerevisiae*) were obtained from Bon - Amour. Pharmacy Limited, Lagos. The betaine and ß-glucan in powder form were supplemented at different doses for each treatment feed and were diluted into 100 ml of warm water to form a solution. The solution was sprayed onto the pelleted feed. Fish oil 15ml/kg feed) was added to preserve the betaine and ß-glucan. The feed was prepared in one kg each time. The feed was mixed by a feed mixer machine to achieve homogenously, pelleted using a pelleting machine, air-dried and kept at room temperature until feeding. Experimental feed was prepared byco - supplementation of betaine and ß - glucan at different dose into formulated feed with 40% crude protein as recommended by Ali *et al.*(2024) at 0.0g/100g (BBG0 i.e. control), 0.325g/100g (BBG1), 0.75g/100g (BBG2), 1.125g/100g (BBG3) and 1.50g/100g feed i.e. (BBG4) as shown in Table 1.

Table 1. Ingredient % (g/100g) of Formulated Feed with Betaine and ß-Glucan Co-Supplementation

Ingredients (%)	BBG0	BBG1	BBG2	BBG3	BBG4
Fish meal	20.00	20.00	20.00	20.00	20.00
Soybean Meal	21.50	21.50	21.00	21.00	21.00
GNC meal	23.00	22.625	22.75	22.375	22.00
Yellow maize	30.00	30.00	30.00	29.75	30.00
Betaine/ß - glucan	**0.00**	**0.373**	**0.75**	**1.125**	**1.50**
Fish oil	1.00	1.00	1.00	1.00	1.00
Vegetable oil	1.00	1.00	1.00	1.00	1.00
Starch	1.00	1.00	1.00	1.00	1.00
Lysine	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
*Vitamin/premix	1.00	1.00	1.00	1.00	1.00
Salt	0.50	0.50	0.50	0.50	0.50
Cr_2O_3	0.50	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00	100.00

Source: Ali (2022).
Key: BBG0 - Betaine and ß - glucan (0.0g/100g), BBG1 - Betaine and ß - glucan (0.375g/100g), BBG2 - Betaine and ß - glucan (0.75.g/100g), BBG3 - Betaine and ß - glucan (1.125g/100g), BBG4 - Betaine and ß - glucan (1.5g/100g).

Experimental Design

The *C. gariepinus* fingerlings were cultured in fifteen (15) rectangular white plastic tanks (flow-through system) with a water holding capacity of one thousand litres (1,000 L) each in a completely randomised design (CRD). Each tank was washed thoroughly with salt, filled to just a little over 1/3 (350 litres) capacity, and stocked with twenty (20) fingerlings of *C. gariepinus* with a mean initial weight of (10.0±0.00 g). The *C. gariepinus* fingerlings were fed the experimental feed at 3% body weight two (2) times daily for twelve (12) weeks. The quantity of feed was adjusted accordingly after every two (2) weeks of sampling for growth performance and survival rate (mean body weight and mortality). Water temperature, pH, dissolved oxygen, and ammonia were measured at the beginning of the experiment, and then weekly throughout the experiment. Water temperature, dissolved oxygen, and pH were measured using the Horiba U-22 XD multi-parameter water quality checker, while ammonia was measured using a freshwater aquaculture test kit (Model AQ-2, Code 3633-03, Lamotte U.S.A.).

Determination of Serum Biochemistry

Blood samples were collected from three (3) fish at the start and again from three (3) fish from each treatment and the control at the end of the feeding trial, following the method described by Dauda *et al.* (2023) for serum biochemical analysis. The fish blood samples were analysed at the haematology laboratory of the Specialist Hospital Gombe, Gombe State, for serum biochemical analysis. Serum biochemical composition, such as total protein (TP), albumin, globulin, the albumin/globulin (A: G) ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, potassium, sodium, and chloride, were determined.

Blood collection and preservation

A 2ml plastic syringe needle was inserted at the ventral midline just posterior to the anal fin of the fish at an angle of 450 until it penetrated the caudal vessel lying between adjacent haemal arches. This was accomplished by inserting the needle until it stopped at the backbone. Blood was then drawn slowly into the syringe and preserved in labelled ethylenediaminetetraacetate (EDTA) bottles.

Serum collection and preservation

The non-heparinised blood samples were centrifuged at 3000 rpm for 10 minutes to extract serum as described by Dauda *et al.* (2023). The serum samples were stored at -80°C for the biochemical analysis.

Serum biochemical composition

Total serum protein was determined by the biuret method, albumin content was estimated by the bromocresol green binding method, globulin content was calculated by subtracting albumin values from serum, while Total protein and albumin/globulin ratio was estimated by dividing albumin values by those of globulin Abdel - Hay *et al.*(2021), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined calorimetrically as described by Dauda *et al.* (2023), while the glucose, potassium, sodium chlorine concentrations were all determined by using flame photometer (Elezuo, 2016).

Statistical Analysis

Data obtained from the research were statistically analysed using one-way analysis of variance (ANOVA) at P = 0.05, where significant differences were detected; mean values were separated using least significant difference (LSD).

RESULTS

The effects of betaine and ß-glucan co-supplementation on serum biochemistry of *C. gariepinus* fingerlings are presented in Table 2. Initial Total serum protein value was 4.00±0.6 g/dL. This increased in all the fish fed feed with betaine and ß-glucan co-supplementation and the control feed (BBG0) final values. The Total serum protein values ranged from 4.20±0.4 to 4.75±0.3 g/dL; the highest Total serum protein value, 4.75±0.3 g/dL, was recorded from fish fed feed BBG2, while the least Total serum protein value, 4.20±0.4 g/dL was recorded from the fish fed the control feed (BBG0). Initial serum albumin value was 2.50±0.1 g/dL. This increased in all the fish fed feed with betaine and ß-glucan co – supplementation and the control feed (BBG0) final values. The serum albumin values range from 2.60±0.1 to 2.75±0.1 g/dL; the highest serum albumin value, 2.75±0.1 g/dL, was recorded from fish fed feed BBG2, while the least serum albumin value, 2.60±0.1 g/dL was recorded from the fish fed the control feed (BBG0). Initial serum globulin value was 1.50±0.01 g/dL. This increased in all the fish fed feed betaine and ß – glucan co – supplementation and the control feed (BBG0) final values. The serum globulin values ranged from 1.60±0.07 – 2.00±0.02 g/dL, the highest serum globulin value, 2.00±0.02 g/dL, was recorded from fish fed feed BBG2, while the least serum globulin value, 1.60±0.07 g/dL was recorded from the fish fed the control feed (BBG0). The initial serum albumin/globulin (A: G) ratio value was 1.67±0.4. This decreased in all the fish fed feed with betaine and ß – glucan co – supplementation and the control feed (BBG0) final values. The serum A: G ratio values ranged from 1.38±0.3 to 1.63±0.4, the highest serum A : G ratio value, 1.63±0.4 was recorded from fish fed the control feed (BBG0), while the least serum A: G ratio value, 1.38±0.3was recorded from the fish fed feed BBG3.

The initial serum aspartate aminotransferase (AST) value was 25.10±2.0 U/L. This increased in all the fish fed feed with betaine and ß – glucan co – supplementation and the control feed (BBG0) final values. The serum AST values ranged from 32.00±1.9 – 32.90±3.7 U/L; the highest serum AST value, 32.90±3.7 U/L, was recorded from fish fed feed BBG4, while the least serum AST value, 32.00±1.9 U/L was recorded from the fish fed feed BBG2. Initial serum alanine aminotransferase (ALT) value was 31.70±1.5 U/L. This increased in all the fish fed feed with betaine and ß – glucan co – supplementation and the control feed (BBG0) final values. The serum ALT values ranged from 53.70±2.1 – 55.00±3.5 U/L; the highest serum ALT value, 55.00±3.5 U/L was recorded from fish fed the control feed (BBG0), while the least serum ALT value, 53.70±2.1 U/L was recorded from the fish fed feed BBG2. Initial serum glucose value was 298.20±15 mmol/L. This increased in all the fish fed feed with betaine and ß – glucan co – supplementation and the control feed (BBG0) final values. The serum glucose values ranged from 304.00±22 – 320.00±11 mmol/L, the highest serum glucose value, 320.00±11 mmol/L, was recorded from

fish fed control feed (BBG0), while the least serum glucose value, 304.00±22 mmol/L was recorded from the fish fed feed BBG2. The initial serum potassium value was 5.13±0.9 mmol/L. This decreased in all the fish fed feed with betaine and ß - glucan co - supplementation and the control feed (BBG0) final values. The serum potassium values ranged from 2.98±0.2 - 3.74±0.3 mmol/L; the highest serum potassium value, 3.74±0.3 mmol/L, was recorded from fish fed feed BBG4, while the least serum potassium value, 2.98±0.2 mmol/Lwas recorded from the fish fed feed BBG1. Initial serum sodium value was 133.60±12.0 mmol/L. This increased in all the fish fed feed with betaine and ß - glucan co - supplementation and the control feed (BBG0) final values. The serum sodium values ranged from 136.30±11.5 - 140.10±11.4 mmol/L; the highest serum sodium value, 140.10±11.4 mmol/L was recorded from fish fed feed BBG4, while the least serum sodium value, 136.30±11.5 mmol/L was recorded from the fish fed the control feed (BBG0). The initial serum chlorine value was 104.30±8.9 mmol/L. This increased in all the fish fed feed with betaine and ß - glucan co - supplementation and the control feed (BBG0) final values. The serum chlorine values ranged from 107.30±10.2 - 110.70±6.7 mmol/L; the highest serum chlorine value, 110.70±6.7 mmol/L, was recorded from fish fed control feed (BBG0), while the least serum chlorine value, 107.30±10.2 mmol/L, was recorded from the fish fed feed BBG3. However, there was no significant difference($p>0.05$) between the Total serum protein, albumin, globulin, albumin/globulin (A : G) ratio, aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose, potassium, sodium and chlorine values recorded from the fish fed feed with betaine and ß - glucan co - supplementation at different doseas compared to the control feed (BBG0).

Table 2: Effects of Betaine and ß - Glucan Co - Supplementation on Serum Biochemistry of *C. gariepinus*) Fingerlings

Parameters	Initial	BBG 0	BBG 1	BBG 2	BBG 3	BBG 4
TP (g/dL)	4.00±0.6 ^{a}	4.20±0.4 ^{a}	4.40±0.7 ^{a}	4.75±0.3^{a}	4.55±0.3 ^{a}	4.50±0.6 ^{a}
A (g/dL)	2.50±0.1^{a}	2.60±0.1^{a}	2.70±0.3^{a}	2.75±0.1^{a}	2.65±0.1^{a}	2.70±0.2^{a}
Gl (g/dL)	1.50±0.01^{b}	1.60±0.07^{b}	1.70±0.03^{b}	2.00±0.02^{b}	1.90±0.01^{b}	1.80±0.05^{b}
A: G ratio	1.67±0.4^{c}	1.63±0.4^{c}	1.58±0.7^{c}	1.38±0.3^{c}	1.40±0.2^{c}	1.50±0.3^{c}
AST (U/L)	25.10±2.0^{a}	32.80±3.4^{b}	32.20±4.7^{b}	32.00±1.9^{b}	32.60±1.8^{b}	32.90±3.7^{b}
ALT (U/L)	31.70±1.5^{b}	55.00±3.5^{c}	54.30±2.8^{c}	53.70±2.1^{c}	54.60±2.2^{c}	54.90±2.1^{c}
G (mmol/L)	298.20±15^{a}	320.00±11^{b}	313.00±21^{b}	304.00±22^{b}	312.00±12^{b}	310.00±18^{b}
K (mmol/L)	5.13±0.9^{b}	3.01±0.7^{c}	2.98±0.2^{c}	3.06±0.2^{c}	3.38±0.2^{c}	3.74±0.3^{c}
Na (mmol/L)	133.60±12.0^{a}	136.30±11.5^{a}	137.80±10.9^{a}	139.60±12.0^{a}	138.40±10.6^{a}	140.10±11.4^{a}
Cl (mmol/L)	104.30±8.9^{a}	110.70±6.7^{a}	108.70±10.0^{a}	107.60±9.8^{a}	107.30±10.2^{a}	107.40±10.8^{a}

Mean values in each row with similar superscripts are not significantly different ($p>0.05$).
Key: TP: Total protein; A: Albumin; Gl: Globulin; G: Glucose; K: Potassium; Na: Sodium; Cl: Chlorine; BBG0 - Betaine and ß - glucan (0.0g/100g), BBG1 - Betaine and ß - glucan (0.375g/100g), BBG2 - Betaine and ß - glucan (0.75.g/100g), BBG3 - Betaine and ß - glucan (1.125g/100g), BBG4 - Betaine and ß - glucan (1.5g/100g).

DISCUSSION

Total serum proteins play an important role in the transport of different substances, the defense of the organism against pathological agents and osmotic regulation (Popoola and Famuagun, 2017). Total serum protein value increased from an initial value of 4.00 g/dL to the final values, which ranged from 4.20 - 4.75 g/dL. These values were comparable with the values, 4.30 - 4.70 g/dL reported by Elezuo (2016) for *C. gariepinus* fingerlings fed mechanically extracted almond kernel meal - based diet. The highest increased in the Total serum protein value observed from the fish fed feed BBG2 could be as a result of the high quality of the feed, and also due to the higher feed intake by the fish and higher feed efficiency ratio (FER).

Albumin is a component of serum protein which plays an important role in the maintenance of blood glucose in fish (Adesina, 2017). Serum albumin value increased slightly from an initial value of 2.50 g/dL to the final values, which ranged from 2.60 - 2.75 g/dL. These values were comparable with the values,2.35- 3.05g/dL, reported by Abdullahi *et al.* (2018) for *C. gariepinus* juveniles fed fermented *Jatropha curcas* kernel meal - based diets. Similarly, to the Total serum protein, the lower value of serum albumin observed in the control feed (BBG0) might be as a result of low feed intake and high feed conversion ratio.

Serum globulin is a component of serum protein (Elezuo, 2016). Serum globulin value increased from an initial value of 1.50 g/dL to the final values, which ranged from 1.60– 2.00 g/dL. These values were lower than the values, 1.60 – 3.10 g/dL, reported by Adesina (2017) for *C. gariepinus* juveniles fed mechanically extracted sunflower (*Helianthus annuus*)seed meal. The increased in serum globulin values of fish populations recorded from this study indicates a stronger innate immunity and was in consistent with the findings of Huong *et al.* (2016) who reported an increase in serum globulin for striped catfish (*P. hypophthalmus*) fed feed supplemented with β – glucan.

Serum albumin/globulin (A: G) ratio value decreased from an initial value of 1.67 to the final values, which ranged from 1.38 – 1.63. These values were higher than the values, 1.13 – 1.50, reported by Elezuo (2016) for *C. gariepinus*.A lower A: G ratio in the serum is an indication that there is a greater globulin concentration in the Total protein compared to the albumin content, which was the situation in the present study. However, since the gamma globulin fraction constitutes the largest part of the overall globulin content, it could be concluded that betaine and β – glucan co – supplementation have stimulatory effects on the serum biochemical indices of the *C. gariepinus* and hence the immune response. The A: G ratio values recorded from the fish fed feed BBG2, BBG3 and BBG4 were within the normal ranged values of 1.2 - 1.5 reported by Elezuo (2016), while A: G ratio values of 1.58 recorded from BBG1 and 1.63 from BBG0werehigher the normal ranged.

The aminotransferase enzyme is an enzyme that catalyses the transamination reaction (Okey *et al.*,2022). There are two types of serum transaminase enzymes, namely aspartate aminotransferase (AST) enzyme and alanine aminotransferase (ALT) enzyme. The main source of AST enzyme is in the liver, whereas ALT enzyme is found in tissues, especially in the heart, skeletal muscle, kidney and brain (Hastuti and Subandiyono, 2018).

Serum AST value increased from an initial value of 25.10 U/L to the final values, which ranged from 32.00 – 32.90 U/L. These values were lower than the values, 53.49 –59.29 U/L, reported by Okey *et al.* (2022) for *C. gariepinus* juveniles fed sesame seed meal as a replacer for soybean meal, and higher than the values, 21.33 – 51.33 U/L, reported by Adesina (2017) for the same species. Elezuo (2016) reported that the recommended ranged values for ALT were 10 U/L – 55 U/L.A decrease in serum AST values was observed in all the fish fed feed with betaine and β – glucan co – supplementation, with the exception of BBG4, as compared to the control feed (BBG0 could be attributed to the dietary amino acid utilisation by fish for growth or high demand for energy. Analyses of serum AST values give proper insight into the status of hepatocytes and diseases, which could be damaging to the liver caused by the reactive oxygen species generated (Ndatsu *et al.*, 2022). However, all the values of serum AST evaluated were within the recommended ranged, which signifies the sound healthy status of the fish.

Serum ALT value increased from an initial value of 31.70 U/L to the final values, which ranged from 53.70 U/L– 55.00 U/L. These values were lower than the values, 65.38 – 73.26 U/L reported by Okey *et al.* (2022) for *C. gariepinus* and higher than the values,11.24–22.43 U/L reported by Ndatsu *et al.* (2022) for *C. gariepinus* juveniles fed fish meal containing different inclusion levels of *Sesame indicum* seed. Elezuo (2016) stated that the recommended ranged of values for ALT was 7 U/L – 56 U/L. An increase in serum ALT values observed in the fish fed feed with betaine and β – glucan co – supplementation as compared to the control feed (BBG0 indicated the high rate of phosphorylation and transport of substances across the cell membrane that may be leading to elevated kidney detoxification effects, elevations of the serum ALT may reflect an alteration in the pathway ways of biosynthesis and mixed – function oxidase (Ndatsu *et al.*, 2022).

Glucose levels are used as an indirect stress indicator in fish, since high stress levels in fish are reflected in higher cortisol amounts in the circulating blood, resulting in corresponding higher glucose levels, thereby increasing susceptibility to disease (Sánchez – Martínez *et al.*, 2017). Serum glucose value increased from an initial value of 298.20 mmol/L to the final values, which ranged from 304.00 -

320.00mmol/L. These values were comparable with the values, 307.00 – 361.00 mmol/L reported by Elezuo (2016) for *C. gariepinus* and significantly higher than the values, 47.00 – 81.33 mmol/L reported by Adesina (2017) for the same species. A rapid secretion of glucocorticoids and catecholamines from the adrenal tissue of the fish fed control feed (BBG0) due to some anti - metabolites might enhance the process of glycolysis, which causes an increase in the serum glucose value. The decrease in serum glucose values observed in the fish fed feed with betaine and β – glucan co – supplementation as compared to the control feed (BBG0)signify the efficacy of the betaine and β – glucan co – supplementation in decreasing the serum glucose values in this research which corroborates the findings of Huong *et al.*(2016) who reported a decrease in serum glucose values for striped catfish (*P. hypophthalmus*) fed feed supplemented with β - glucan at 1.00g/kg.

Electrolytes such as potassium, sodium and chlorine are molecules found throughout the blood tissues and cells of fishes, including *C. gariepinus* (Ike – Obasi and Ukazu, 2018). These molecules which could be either positive or negative ions conduct electric current and help to balance pH and acid base levels in the fish, they also facilitate the passage of fluid between and within cells through a process known as osmosis and also play a part in regulating the functioning of neuro-muscular, endocrine and excretory system of fishes, electrolyte concentration is indicative of the ability of the fish to osmoregulation (George and Uedeme – Naa, 2020).

Serum potassium value decreased from an initial value of 5.13 mmol/L to the final values, which ranged from 2.98 – 3.74 mmol/L. These values were lower than the values,6.92 – 11.60 mmol/L reported by George and Uedeme – Naa (2020) for *C. gariepinus* juveniles in response to treatment with detergent (linear alkylbenzene sulfonate) and the values, 6.83 – 13.40 mmol/L reported by Ike – Obasi and Ukazu (2018) for *C. gariepinus* juveniles treated with paraquat dichloride. These values were within the recommended ranged values, 2.30 – 5.2 mmol/L reported by Elezuo (2016). This indicated that betaine and β – glucan co – supplementation into the feed does not have any negatively effect on the serum potassium values.

Serum sodium value increased from an initial value of 133.60 mmol/L to the final values, which ranged from 136.30 – 140.10 mmol/L. These values were lower than the values, 157.33 – 259.02 mmol/L reported by Ike – Obasi and Ukazu (2018) for *C. gariepinus* and the values, 111.00 – 185.50 mmol/L reported by George and Uedeme – Naa (2020) for *C. gariepinus*. These values were within the normal ranged values, 130.00 – 150.00 mmol/L reported by Elezuo (2016). This signifies that betaine and β – glucan co – supplementation into the feed at different doses employed in this research stabilised the serum sodium values of the fish to the optimal levels.

Serum chlorine value increased from an initial value of 104.30 mmol/L to the final values, which ranged from 107.30 – 110.70 mmol/L. These values were comparable with the values,111.50 – 114.10 mmol/L reported by Elezuo (2016) for *C. gariepinus* fingerlings fed raw extracted almond kernel meal – based diet, but lower than the values, 169.25 – 298.25 mmol/L reported by George and Uedeme – Naa (2020) for *C. gariepinus*. The values recorded from the fish fed feed with betaine and β – glucan co – supplementation at different dose employed in this researchwere within the recommended ranged values, 98 – 108 mmol/L reported by Elezuo (2016), while the value of 110.70 mmol/L recorded from the fish fed control feed (BBG0) was higher than the normal ranged, this indicated that the feed with betaine and β – glucan co – supplementation at different dose was suitable in maintaining the fish serum chlorine values.

CONCLUSION

Findings from this research indicated that betaine and β – glucan co – supplementation at different dosei.e.0.325g/100g, 0.75g/100g, 1.125g/100g and 1.50g/100g feedemployed in this study did not result in statistically significant ($p>0.05$) changes in the serum biochemistry, but had positive effect in the physiology of the *C. gariepinus* fingerlings by

increasing the total serum protein values and lowering the serum glucose values as compared to the control feed which has 0.0g/100g feed betaine and ß – glucan co – supplementation. However, the best physiological response, i.e. highest Total serum protein value (4.75±0.3 g/dL) and the least serum glucose value (304.00±22 mmol/L) were both recorded from betaine and ß – glucan co – supplementation at the dose of 0.75g/100gfeed. Therefore, further research may be required to assess the suitability of betaine and ß – glucan co – supplementation at higher dose, its correlation with the various biological impacts, and the possible implications for human consumers.

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