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Some Biochemical Indices of Broiler Chickens Fed Graded Levels of Lysine Supplement, Maiduguri, Nigeria

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Abstract

This research study was conducted on some biochemical indices of broilers chicken fed graded levels of lysine supplement in Maiduguri was carried out to determine their biochemical indices and ascertained the level of inclusion of lysine in poultry feed. Standard operating procedures were followed. 120 day-old broiler chickens were obtained commercially, grouped in to 4 experimental treatments and was randomized complete block designed (T); T_1, T_2, T_3 and T_4 with 15 birds per treatment and each with replicates. Brooded for a week with starter feed, experimental diets of which lysine supplement was added to T_2, T_3 and T_4 at 0.02, 0.03 and 0.04 %, but not to T1 as control. Feeds and water were provided ad-libitum, for 4 weeks. Blood samples were obtained through Veni-puncture for serum biochemical indices analyses and estimations by AAS techniques as described by AOAC [1]. Results obtained revealed the values of mean concentration level in mmol / l of; alanine amino transferase ranged from 46.10 to 53.10, albumin (25.02 to 36.00), aspartate amino transferase (36.11 to 68.00), blood urea (2.10 to 3.20), creatinine (1.60 to 2.20), globulin (14.00 to 18.00), glucose (5.71 to 6.01) and total protein ranged from 39.00 to 52.01 respectively. Lysine supplement was found to be effective at 0.02 to 0.03 % of. Both the values fall within the normal range values and the results obtained are in conformity with the works of most authors and researchers.

Keywords: Ad-libitum; Biochemical indices; Broiler chickens; Experimental diet, Serum.

1. Introduction

When dealing with food security, high quality and quantity of animal production for quality protein have to be considered. In the past several decades, a great effort has been taken in advances, in animal nutrition, production practices and genetics have kept animal production in line with a growing large population and increasing the consumer demand for high quality and numerous quantity sources of animal protein globally, particularly birds (Avian); include poultry production, especially broiler chickens.

Birds are natural creatures reared or hunted for useful purpose, belonging to a number of bird groups collectively known as poultry. They are domesticated and managed in the same principles as domestic fowl (avian), e.g., chicken, duck, guinea fowl, turkey, etc., as a means of meeting human nutritional needs as well as improving incomes of farmers and their standards of living [2-4]. Chicken has many advantages over other domesticated animals, of which production is costly and highly demanded. It is also being affected by temperature, environmental diseases and lack of food at certain period of time [5, 6]. Akinde [2], defines the terms poultry as birds that are raised domestically by human being for the purposes of food and other reasons produced freely under intensive management and control.

For many decades, farmers and feed manufacturers have been facing the challenge of effectively reducing the cost of poultry production and produce quality products. Dietary management of energy intake has been reported to decrease the cost of production and improve product quality to a greater extent than the above mentioned factors [7-9]. The addition of fat to diets, besides supplying energy, improves the absorption of fat-soluble vitamins, diminishes the pulverulence, increases the palatability of the rations, and increases the efficiency of the consumed energy [8, 10]. Any poultry producer aims are to feed the chickens with balanced diet at least cost and also generate products that will attract premium prices in order to maximise profit objectively. Strategies for feeding broilers destined for the whole bird market will differ from strategies for broilers destined to be sold as pieces. Furthermore, the nutrient

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intake of fast growing broilers must be carefully controlled to prevent metabolic diseases such as ascites and leg weakness [11, 12].

Aftab, *et al.* [13] and Ahmed, *et al.* [14] stated that, these demands have been achieved with the utilization of high quality feed ingredients such as wheat, maize, soybeans and corn. Poultry diets, typically contains a variety of feedstuff. This is because, no single ingredient is able to supply all the necessary amino acid in the right level. Most feedstuff only indicate the percentage of protein, amino acids, crude protein, dry matter, fibre, minerals, moisture content in a given feed for poultry [3, 15, 16].

In poultry, especially broilers, their body needs lysine for healthy physiological functions. It is a crucial components of protein that play a vital role in helping body tissue grow and recover (repaired wounded out) from damage. Other benefits of lysine include, helping the body to absorb calcium, iron, and zinc, promoting collagen growth, help to produce enzymes, antibodies, and hormones. Lysine is an amino acid use in biological or biosynthesis of proteins [6, 8, 9, 17].

Lysine (L-lysine HCl and L-lysine H_2SO_4) is one of the most critical amino acid required by poultry as essential amino acid for growth and maintenance of weight. Lysine also allows the limiting use for protein bound which is contained in vegetable such as soya beans meals [17-20]. Lysine also play a vital role in growth, as well as the production of carnitine, which turns fats into energy and help lower cholesterol. It also assists in calcium absorption and collagen production. Collagen is responsible for keeping skin, bones, cartilage and tissue healthy. Diets with adequate protein should be supply enough; however, animals with insufficient diets, certain injuries or diseases may benefit from lysine supplementation [5, 17, 21]. Lysine also has some therapeutics uses. It can prevent herpesvirus from growing, and can reduce the security, healing time and recurrence of the virus. Animal form of herpes include; rhino-pneumonitis, rhinotrachuris virus and feline herpesvirus type 1, a common cause of respiratory infection in cats. Lysine is taken as capsules, tablets or liquid, and topically as a cream. Dosage catered to an animals' weight. Researched dosages reflect 12 milligrams per kilogram of body weight, and skin cream application every to 2 hours for up to 11days [2, 3, 18]. Long term doses can cause gall stone or kidney problems, and supplementation should not last more than a year. This is because, lysine increase calcium absorption, monitor your animals' dietary calcium levels. Therefore, consult with an animal science, nutritionist before beginning an animal on lysine [14, 18, 22].

Lysine is one of the essential amino acid in most protein sources to poultry, it is cheap as supplement and can be found in synthetic form to be purchased and easily obtained to meet farmer's need, and is still limiting in most poultry diets. Hence, it is important to conduct a research on it due to the increase in demand for foods, especially protein becomes acute. To meet the demand for animal proteins, the development of animal production, especially in the fast developing and rapidly multiplying areas poultry production becomes necessary The objectives of this study are to determine some of the biochemical indices of broiler chickens fed with grade level of lysine supplement and to ascertain the levels of inclusion of lysine in poultry feed for maximum performance.

2. Methodology

2.1. Study Area

The experimental study was conducted at poultry production unit of teaching and research poultry farm of the Department Animal Health and Production Technology Mohamet Lawan College of Agriculture, Maiduguri, in Jere Local Government Area, Borno State of Nigeria.

Jere is situated below 305 m above sea level, north of Maiduguri. It is located between latitude 110^o 48' – 110^o 58' N and longitude 130^o 06' – 130^o 20' E in the Sudan – savannah transition zone. Jere Bowl covers an area of about 22,000 ha, out of which a gross area of 15,850 ha was identified as suitable for irrigated agriculture. Jere bowl fall within Jere LGA and it shares boundaries with some local government areas; to the northeast shares border with Nganzai and Mafa, while to the north-west and south-east shares border with Maiduguri and Konduga. The study area was selected based on its proximity, accessibility, relevance of the study and familiarity with the environment. Intensive irrigation activities take place all year round at the banks of the river [23, 24],

2.2. Materials

A hygienically clean lighted fit poultry house, and materials used are also cleaned and of highly grade.

2.2.1. Reagents Used

Reagents used in this research study are of analytical grade.

2.2.2. Type of Feeds Used

Commercially obtained starter feed (Chukun brand feeds), Finisher feed (Self - Formulated), commercially obtained Lysine, Izal disinfectant, detergent, Tap water, Deionised water and saw – dust.

2.3. Methods

The following methods were applied and standard operating procedures (SOP) and safety precautions strictly were observed as described by AOAC [1].

2.3.1. Cleaning and Disinfecting the Poultry House

Procedures: - The house was swept and cleaned, traces of cobwebs and dust were swept. The floor was also washed with water and detergent and disinfectant was spread all over the floor. The cleaned and disinfected house

was left opened and all windows were left opened to air dried and ventilated for 3 days. Then after left 3 days and was ready for stocking the day old chicks.

2.3.2. Farm - Formulated Finisher Feed

The starter feed was obtained commercially. The finisher feed was formulated locally by mixing together the calculated amount and weight of the ingredients in grams, thoroughly and homogenously. The feed ingredients were; maize, wheat bran, fish meal, bone meal, common salt, methionine and premix. These were contained in each feed treatments (T_1, T_2, T_3 and T_4) and except the quantity of lysine varies in each of the treatments.

2.3.3. Experimental Stocking and Management

Procedures: - A total of 60 day old broilers chickens were purchased from ECWA, Maiduguri.

They were transported to the poultry house unit of the Mohamet Lawan College of Agriculture, Maiduguri. The method used in this experimental design study was randomized complete block designed. The birds were fed with broiler starter and brooded for the period of four weeks. After brooding, the birds were divided in to four groups, hence experimental treatments (T); T_1, T_2, T_3 and T_4 with 15 birds per treatment and with their replicates each. Then, later experimental diets in which lysine supplement was added to T_2, T_3 and T_4 at 0.02 %, 0.03 %, 0.04 % while lysine supplement was not added to T_1 as control respectively. Feeds and water were provided ad – libitum, for 4 weeks. Now the birds are ready for the table sizes and for the Biochemical indices analysis.

2.3.4. Blood Samples Collection by Using Veni - Puncture

Procedure: - Prior to blood collection, the experimental birds were devoid feeding 3 hours' time. The experimental bird was restrained, and wing vein was located. The vein location was swabbed with cotton wool soaked in 70 % methylated spirit in order to disinfect and provide prominent wing vein. With the used of 5 ml syringe and needle, 5 ml of blood sample was collected and dispensed in to plain blood sample container, screwed capped, labelled and left to stand for several minutes for the blood sample to clot. Within the labelling; the name of the treatment and the replicate, date and time of the blood sample collected. They were parked in to cool chain box and transported by road to the Animal Science Laboratory, Department of Animal Science, Faculty of Agriculture, University of Maiduguri, Maiduguri, Nigeria, for the analysis.

2.3.5. Centrifugation of the Clotted Blood Samples

This was done by separating the serum from the whole clotted sample by using centrifugal force. On arrival to the laboratory, samples were removed from the cool chain box and placed on the working bench in order to cool down to a room temperature.

Procedures: - The clotted blood sample was unscrewed opened and the clotted blood was detached from the wall of the container and screwed capped. It was then inserted in to centrifuge buckets each at opposite direction in order to balance and with equal volume of the samples and cover the lid of the centrifuge machine. The centrifuge machine was then set at 3000 revolutions per minute (rpm) for 5 minutes. The centrifuge machine was on and after 5 minutes was off automatically, was allowed stopped spinning on itself. It was then, the spun clotted blood samples in a container were removed and placed on the working bench. Resting on the working bench, the spun clotted blood sample formed 2 layers; the packed cells at the bottom of the container and the clear supernatant fluid being the serum.

2.3.6. Separation of the Serum Technique

Procedures: - With a plastic pipette, the supernatant (clear serum) was pipetted out and dispensed in to a labelled, cleaned and sterile plain blood container. It was screwed capped and kept for further respective analysis required.

2.3.7. Determinations of Some of the Biochemical Indices of Serum Sample

The blood / serum samples in which biochemical indices such as the total protein, albumin, creatinine, blood urea, globulin, aspartate amino transferase, alanine amino transferase and glucose estimations by their distinctive various techniques and read through atomic absorption spectrophotometer (AAS) techniques were performed as described by AOAC [1].

2.4. Data Analysis

He data obtained in this research study were analysed by using analysis of variance (ANOVA). The mean between the treatment were separated using LSD at 5 % of probability.

2.4.1. Calculated Feed Ingredients

In the experimental feed formulation, the calculated feed ingredients were performed as shown in table 1.

3. Results

The results (data) obtained from this research study were presented in the tables below as follows:

Table 2 Showed the biochemical indices of serum sample obtained from the broilers that were fed with graded levels (treatments T_1, T_2, T_3 and T_4) of lysine measured in millimole per litre (mmol / l). The biochemical indices

measured were; creatinine values ranged from 0.90 to 2.20 mmol / l, blood urea (2.10 to 3.20 mmol / l), total protein (39.0 to 52.01 mmol / l), albumin (25.02 to 36.0 mmol / l), globulin (14.0 to 18.0 mmol / l), aspartate amino transferase (36.11 to 68.00 mmol / l), alanine amino transferase (46.10 to 53.10 mmol / l) and finally glucose ranged from 6.01 to 5.91 mmol / l respectively.

Table-1. Calculated Composition of Feed Ingredients of the Treatments

Food Items	**Weight of Ingredients Per Treatment in Grams (g)**			
	T_1	**T_2**	**T_3**	**T_4**
Maize	53.10	53.10	53.10	53.10
Wheat Bran	11.10	11.10	11.10	11.10
Fish Meal	5.00	5.00	5.00	5.00
Bone Meal	3.00	3.00	3.00	3.00
Salt (Iodide NaCl)	0.25	0.25	0.25	0.25
Premix	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20
Lysine	0.20	0.02	0.03	0.04
Total	**73.1**	**72.92**	**72.93**	**72.94**

Table-2. Serum Biochemical Indices of Broiler Chickens Fed Graded Level of Lysine Supplement

Biochemical Indices (mmol / l)	**Treatment**			
	T_1	**T_2**	**T_3**	**T_4**
Alanine amino transferase	46.10	49.01	48.00	53.10 ^{NS}
Albumin	30.10	36.00	25.02	31.01^{NS}
Aspartate amino transferase	36.11	68.00	42.02	58.10^{NS}
Blood urea	3.20	2.60	3.01	2.10^{NS}
Creatinine	1.60	2.20	1.80	0.90^{NS}
Globulin	18.00	16.00	14.00	15.00^{NS}
Glucose	5.80	5.91	5.71	6.01^{NS}
Total protein	48.01	52.01	39.00	46.0 ^{NS}

Keys: NS = means were not significantly difference (P > 0.05) among treatments.

Figure-1. Showed the calculated Composition of Feed Ingredients in each of the Treatments

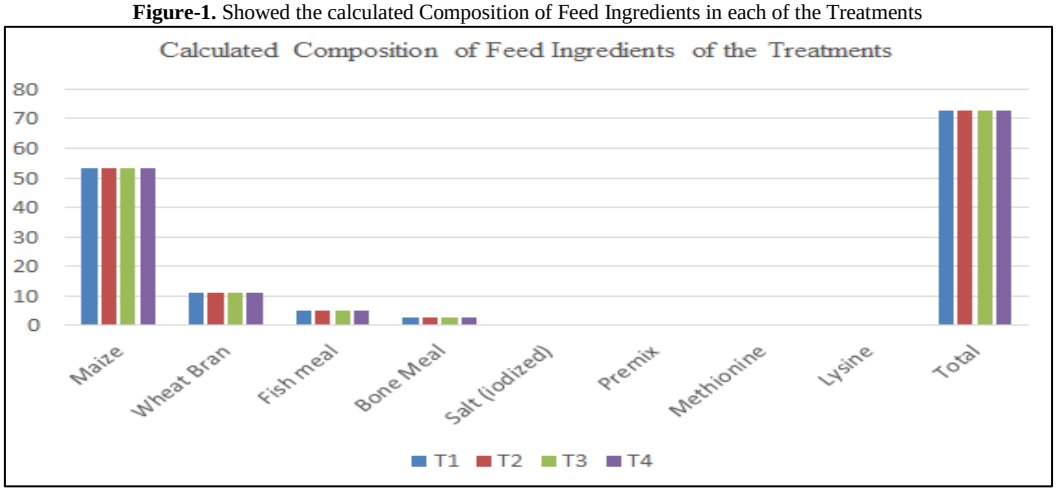
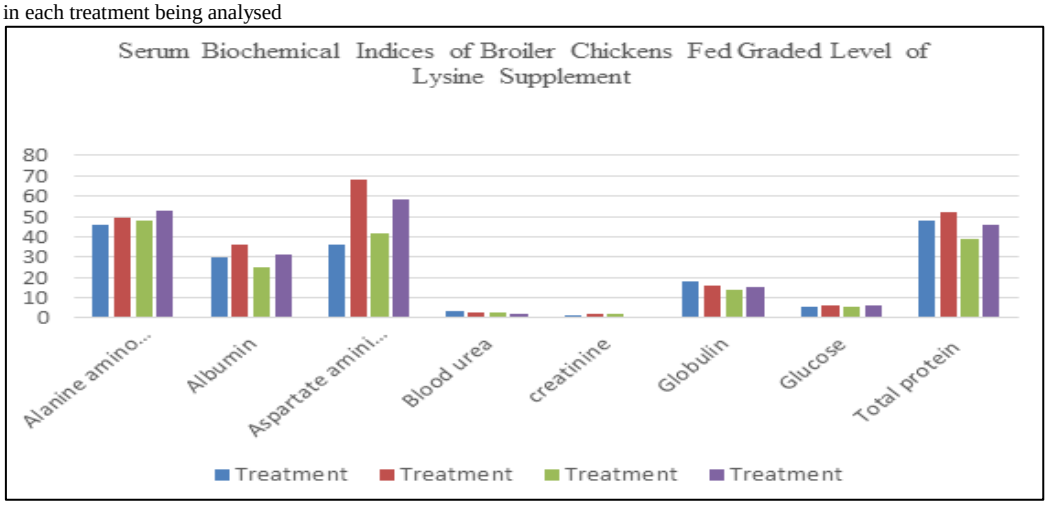


Figure-2. Showed the mean values of concentration levels of some serum biochemical indices of broiler chicken fed graded level of lysine supplement in each treatment being analysed



4. Discussions

The results of the analysis of serum biochemical parameters of the broilers fed with graded level of lysine that were obtained, revealed that in all of the experimental treatments, alanine aminotransferase (ALT) ranged from 46.10 to 53.10 mmol / l, with treatment 1 (T_1) which is been the control that lysine supplement was not added to it, had 46.10 mmol / l. The highest in terms of concentration value obtained in this study was treatment 4 (T_4) had 53.1 mmol / l, followed by treatment 2 (T_2) with 49.01 mmol / l, and the least in concentration value was treatment 3 (T_3) which had 48.00 mmol / l. These values that were obtained are within normal range for *Gallus gallus* specie, as Marcos, *et al.* [21] and Lumeji [25] reported that in most avian species the blood parameter varies from 19 to 50 IU / L. Sequentially, their magnitude in descending order were as follow; $T_4 > T_2 > T_3 > T_1$ respectively. Conclusively, from these results obtained, it revealed that the 3 experimental treatments; T_2 (0.02 %), T_3 (0.03 %,) and T_4 (0.04 %) had more of the concentration value of ALT than the control treatment T_1 (0 %).

With consideration of the analytes (serum biochemical parameters) determined, the results revealed that albumin ranged from 25.02 to 36.00 mmol / l. The highest was experimental treatment 2 (T_2) with the concentration value of the albumin was 36.00 mmol / l, followed by treatment (T_4) with the concentration value of 31.01 mmol / l, then treatment 1 (T_1) which was the control treatment had the value of 30.10 mmol / l. The least was treatment 3 (T_3) with the value of albumin (25.02 mmol / l). Both the values (control treatment and the 3 test treatments) falls within normal values (1.1 – 2.1 gm / dl) as reported by Denise, *et al.* [26]. Therefore, when we arrange the values obtained in descending, sequential order of their magnitude we will have the following; $T_2 > T_4 > T_1 > T_3$. From these results obtained, it was observed that the 3 experimental treatments; T_2 (0.02 %) and T_4 (0.04 %) had more of the concentration value of albumen than the control treatment T_1 (0 %). While T_3 (0.03 %,) had less of the concentration value of albumen than the control treatment T1 (0 %) respectively. In another observation, the serum biochemical analytes of the broiler chicken fed with graded level of lysine determined, the results obtained revealed that aspartate amino transferase (AST) ranged from 36.11 to 68.00 mmol /l. Experimental treatment 2 (T2) had 68.00 mmol /l which is the highest concentration value of the AST obtained. Seconded by treatment 4 (T4) had 58.10 mmol / l, then followed by treatment 3 (T3) with 42.02 mmol / l, and least in term of concentration value was treatment 1 (T1) had 36.11mmol / l, which was the control treatment. Both the values (control treatment and the 3 test treatments) falls within normal values (225 – 499 IU / L) as reported by Denise, *et al.* [26]. Sequentially, their magnitude in descending order were; T2 > T4 > T3 > T1. From these results obtained, it revealed that the 3 experimental test treatments; T2 (0.02 %), T3 (0.03 %,) and T4 (0.04 %) had more of the concentration value of AST than the control treatment T1 (0 %).

Furthermore, the serum biochemical analytes of the broiler chicken fed with graded level of lysine determined, the results obtained revealed that blood urea (BU) ranged from 2.10 to 3.20 mmol /l. Experimental control treatment 1 (T_1) had 3.20 mmol /l, was found to be the highest in concentration value of the BU obtained. Seconded by the test treatment 3 (T_3) which had 3.01 mmol / l, followed by the test treatment 2 (T_2) with 2.60 mmol / l, and least in term of concentration value was treatment 4 (T_4) had 2.10 mmol / l. It was observed that the control treatment; T_1 with 0 % of lysine, had the concentration value of blood urea more than the 3 experimental test treatment; T_2 (0.02 %), T_3 (0.03 %,) and T_4 (0.04 %). When arrange Sequentially, their magnitude in descending order were as follow; $T_1 > T_3 > T_2 > T_4$ respectively.

In addition, the results of the analysis of serum biochemical parameters of the broilers fed with graded level of lysine that were obtained, revealed that in all of the experimental treatments, creatinine ranged from 1.60 to 2.20 mmol / l. The highest was experimental treatment 2 (T_2) with the concentration value of the albumin was 2.20 mmol / l, followed by treatment (T_3) with the concentration value of 1.80 mmol / l, then treatment 1 (T_1) which was the control treatment and no lysine supplement was added to it had the value of 1.60 mmol / l. The least was treatment 4 (T_4) with the value of creatinine (0.90 mmol / l). These results obtained in this research work falls within normal values, and in conformity with report of Sandhu, *et al.* [27] who stated that this catabolite is directly related to increase muscles activity and volume. Younger and older broiler chickens have low levels of blood creatinine. Therefore, when we arrange the values obtained in descending, sequential order of their magnitude, will be the following; $T_2 > T_1 > T_3 > T_4$. From these results obtained, it was observed that the 2 experimental treatments; T_3 (0.03 %) and T_4 (0.04 %) had less creatinine concentration level than the control treatment T_1 (0 %). While T_2 (0.02 %,) had more of the concentration value of creatinine than the control treatment T_1 (0 %) respectively

It was observed that the results of the analysis of serum biochemical parameters of the broilers fed with graded level of lysine supplement that were obtained, revealed that in all of the experimental treatments, globulin ranged from 14.00 to 18.00 mmol / l. Experimental control treatment 1 (T_1) is been the control where lysine supplement was not added to it, had the highest concentration value of 18.00 mmol / l, followed by treatment 2 (T_2) with 16.00 mmol / l, and then test treatment 4 (T_4) was with the value of 15.00 mmol / l. The least in concentration value was treatment 3 (T_3) which had 14.00 mmol / l of globulin. These values that were obtained are within normal range for *Gallus gallus* specie that is 0.5 to 1.8 g / dl, as cited by Thrall [28], who reported that globulin did not vary with age. Sequentially, their magnitude arrange in descending order were as follow; $T_1 > T_2 > T_4 > T_3$ respectively. Conclusively, from these results obtained, it revealed that the control treatment T_1 (0 %) had more of the concentration value of globulin than the experimental treatments; T_2 (0.02 %), T_3 (0.03 %,) and T_4 (0.04 %).

Never the less, it was observed that the results of the serum biochemical analytes in the broilers fed graded level of lysine supplement, revealed that in all of the experimental treatments, glucose ranged from 5.71 to 6.01 mmol / l. The highest was experimental test treatment 4 (T_4) with the concentration value of the glucose was 6.01 mmol / l, followed by test treatment 2 (T_2) with the concentration value of 5.91 mmol / l, then treatment 1 (T_1) which was the control treatment and no lysine supplement was added to it had the value of 5.80 mmol / l. The least was test

treatment 3 (T_3) with the value of (5.71 mmol / l (glucose). These results obtained in this research work falls within normal values and in conformity with the works of most researchers and authors. The greatest glucose occurred at older days (age), which is probably due to stressing moment during the blood sampling as Thrall [28] reported. Broilers chicken had blood glucose levels within the normal range (200 to 500 mg / dl) as reported by Marcos, *et al.* [21]. When we arrange the values obtained in descending order of magnitude, it will be the following; $T_4 > T_2 > T_1 > T_3$. From these results obtained, it was observed that the 2 experimental treatments; T_2 (0.02 %) and T_4 (0.04 %) had more glucose concentration level than the control treatment T_1 (0 %) and T_3 (0,03 %). While T_1 (0 %,) had more of the concentration value of glucose than the control treatment T_3 (0.03 %).

Finally, in consideration of the analytes (serum biochemical parameters) determined, the results revealed that total protein within the experimental treatment ranged from 39.00 to 52.01 mmol / l. The highest was experimental treatment 2 (T_2) with the concentration value of the albumin was 52.01 mmol / l, followed by control treatment 1 (T_1) with the concentration value of 48.01 mmol / l, then treatment 4 (T_4) had the value of 46.00 mmol / l. The least was treatment 3 (T_3) with the value of total protein (39.00 mmol / l). Both the values (control treatment and the 3 test treatments) falls within normal values (2.5 – 4.5 g / dl) as cited by Aletor, *et al.* [29], Alleman and Leclercq [30], Thrall [28]. When we arrange the values obtained in sequential descending order of their magnitude, we will have the following; $T_2 > T_1 > T_4 > T_3$. From these results obtained, it was observed that the 2 experimental treatments; T_3 (0.03 %) and T_4 (0.04 %) had less of the concentration value of total protein than the control treatment T_1 (0 %). While T_2 (0.02 %,) had more of the concentration value of the total protein than the control treatment T_1 (0 %) respectively. At 0.02 to 0.03 % of lysine supplement was found to be effective these results that were obtained in these research work supports the works of most researchers and authors.

5. Conclusion

The results of the analysis of serum biochemical parameters of the broilers fed with graded level of lysine revealed that in all of the experimental treatments, alanine aminotransferase ranged from 46.10 to 53.10 mmol / l, albumin (25.02 to 36.00 mmol / l), aspartate amino transferase (36.11 to 68.00 mmol /l), blood urea (2.10 to 3.20 mmol /l), creatinine (1.60 to 2.20 mmol / l), globulin ranged (14.00 to 18.00 mmol / l), glucose (5.71 to 6.01 mmol / l), and total protein within the experimental treatment ranged from 39.00 to 52.01 mmol / l and at 0.02 to 0.03 % of lysine supplement was found to be effective. Both the values (control treatment and the 3 test treatments) falls within normal values and the results obtained are in conformity with the works of most authors and researchers.

Recommendations

Based on the findings of this research study, the following recommendations were made:

Further intensive research study in study area and its environs on the blood or serum or both biochemical and haemocytometry indices or parameters of avian species are highly recommended.

Also this research study recommends that further study on normal – range values of avian (especially, *Gallus gallus* species) haematologic and serum biochemical reference interval should be carry out in the said study area and if conducted it will serve as baseline reference study.

Furthermore, there are great needs for fund in order to carry out such research study. Hence, this research study recommends that the Governments through its' ministries, parastatals, institutions, agencies, bureaus, etc., Non-Governmental Organisations, Stake-holders, Companies, Industries and Individual stakeholders, etc., should raise funds for the purpose of sponsoring such research for benefit of mankind and animals in order to ascertain the physiological and anatomy clinically and health status of avian, their blood need to be analysed for haematological (Haemocytometry) and haematochemistry (Serum Biochemical) parameters and their indices for healthier, excellent quality and plentiful quantity of poultry products.

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