

Intestinal mucosa development and blood profile of broiler chickens administered bovine testicular fluid

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ABSTRACT: A seven-week experiment was carried out to determine the effect of bovine testicular fluid on intestinal mucosa development, haematological variables and serum biochemical indices of broiler chickens. A total of 125 unsexed day-old Abor acre chicks were randomly allotted into five treatments, with each treatment having five replicates with five birds per replicate. They were fed a commercial broiler diet for seven weeks of the experiment. Different concentrations of the bovine testicular fluid per liter of drinking water were offered to the experimental birds at free choice, the birds in treatment 1 served as the control and they were offered ordinary drinking water while birds in treatments 2, 3, 4 and 5 were offered 25, 50, 75 and 100 ml of bovine testicular fluid per liter of water, respectively. The feed and water intake of each bird were measured on daily basis, while the average weight of the birds was measured on weekly basis. On day 21 and 49, blood samples were collected from the birds to determine the haematological and serum biochemical indices. Two birds from each replicate were sacrificed on day 49 and sample of ileum were taken for intestinal mucosal examination after processed into permanent slides. The results of the blood indices obtained on days 21 and 49 indicated that the blood parameters were within the normal physiological range for chickens and without evidence of organ damage. The villus height, villus width, crypt depth, crypt height, and mucosa thickness showed that the bovine testicular fluid increased the absorptive capacity of the intestinal mucosa surface area and their growth. It is recommended that bovine testicular fluid at 100 ml/L improved intestinal mucosa development and absorption capacity of broiler chickens and enhanced their growth without deleterious effect on their health status.

Keywords: Bovine testicular fluid, broiler chickens, haematology, serum biochemistry.

INTRODUCTION

The use of antibiotics as a growth promoter in the livestock industry has been found as a major threat to the commercial poultry industry and human health. Evidence has shown that the residual effect may compromise the health status of the end users (humans). The consequences of which led to the recent ban on the use of such synthetic growth promoter in the production of livestock in developed countries of the world (Hughes and Heritage, 2004). The implication of the ban in the use of antibiotic growth-promoters has led to the manipulation of feed ingredients, by the feed mill industry in order to improve and balance up their profit margin (Adejinmi et al., 2015). The implication of which has led to the poor turnover of broiler chickens and low consumption of adequate animal protein by the human population of the

country. Poultry production is the best livestock sector that can easily make available sufficient animal protein needed to survive and maintain the human body growth and development. This is because it is very cheap, affordable, and has a short gestation period compared to sheep, goat and cattle. To provide and improve the availability and the daily consumption of animal protein by the increasing human population of Nigeria, there is a need to focus our research on the growth-promoting potentials of bovine testicular fluid, which can be obtained from the testes of a matured bull; a waste product readily available in abattoirs. This natural growth-promoter could be used to replace antibiotic growth-promoter in broiler chicken production, as it contains crude testosterone – a growth-enhancing additive.

The bovine testicular fluid, which consists mainly of testosterone as the chief hormone (Egbunike et al., 2008, Adejinmi et al., 2015; Ewuola and Olujimi, 2019), could improve the growth performance and welfare of broiler chickens by synergizing with other hormones to enhance the feed efficiency thereby improve weight gain (William et al., 1992; Annamaria, 2012; Ewuola and Olujimi 2019), and also improve the performance of various organs and system such as digestive system and the heart function (Muthusamy et al., 2011). According to Kitt et al. (2001), villi and the crypt are the major region within the small intestine in which most of the feed digestion and absorption of the feed nutrients takes place. However, the implication of testicular fluid on the blood profile when used as a growth promoter in broiler chickens and the mechanism by which the feed nutrients is been absorbed by the villus to influence growth development are not clear. Therefore, this study was designed to examine the effects of the bovine testicular fluid on the blood profile and intestinal mucosa development in broiler chickens.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Poultry Research Unit of the Teaching and Research Farm, University of Ibadan, Ibadan, Oyo State, Nigeria.

Preparation of the bovine testicular fluid

Testicular fluid was obtained from bulls testes via extraction and centrifugation. Fresh bull testes from healthy bulls were obtained from a reputable abattoir in Ibadan. The tunica albuginea of the testes was removed and the testis was crushed. The crushed testes were homogenized in a dilution of 30 ml of water per gram of testis. An aqueous homogenate was obtained afterwards. The aqueous homogenate was centrifuged at 1,500 rpm (revolution per minute) for 5 minutes (Ewuola and Olujimi, 2019). The result was a testicular fluid (containing crude testosterone). The crude testosterone extract in form of testicular fluid was included in the bird's water at graded levels. The testicular fluid was stored in the refrigerator at 4°C until when needed every day.

Experimental design and procedure

A total number of 125 unsexed day-old chicks (Abor acre) were used for this study. The birds were randomly distributed into 5 treatments, each with 5 replicates, and 5 chicks per replicate. The birds were brooded on wood shavings and fed on a commercial broiler starter diet, containing 22.8% crude protein and 3035.39 kcal/kg

metabolizable energy, for three weeks; and, finisher diet, containing 20.7% crude protein and 2834.80 kcal/kg metabolizable energy, for four weeks. The birds in the treatment one, which was the control were offered only ordinary drinking water while the treatments 2, 3, 4 and 5 birds were offered 25, 50, 75 and 100 ml of the bovine testicular fluid per liter of the drinking water, respectively at free choice for the seven weeks of the study. The feed intake and water intake of the birds were recorded on daily basis, while all the necessary vaccinations and medication schedule were done as recommended for the strains of broiler chickens used in this study. The completely randomized design was adopted in distributing the birds into experimental treatments.

Haematological and serological characteristics of the experimental birds

The blood samples were collected into two vials from each of the 5 treatments group per replicate. The birds were bled by the jugular vein using hypodermic needles with a syringe. One vial contained ethylene diamine-tetra-acetic acid as an anti-coagulant for haematological assessment, while the other vial for serum collection had no anti-coagulant for the serum analysis of the blood sample.

Determination of haematological parameters

The haematological parameters assessed include; packed cell volume, white blood cell, red blood cell, haemoglobin, platelets, blood constants, as described in Ewuola and Egbunike (2008).

Determination of serum biochemical parameters

The serum biochemical parameters assessed include; total protein, cholesterol, globulin, albumin, albumin/globulin ratio, creatinine, alanine amino transferase, aspartate amino transferase and alkaline phosphatase, as described by Ewuola and Egbunike (2008).

Intestinal mucosa development assessment

Samples of ileum were taken for examination from 2 average broilers (randomly selected) from each replicate/treatment (n = 50) and processed into permanent slides. Sections of 5 to 8 mm thickness were taken from each sample and fixed in 10% formalin solution and absolute alcohol and embedded in paraffin. Sections were cut, mounted on slides and were stained with the HE method as described by Scheuer and Chalk (1986).

Morphometric examinations of the villus height and width, as well as crypt depth, crypt height, and mucosa thickness were done by light microscopy at a magnification of X400 as described by Djolai et al. (1998) and Ewuola et al. (2011).

Data analysis

Data were analyzed using ANOVA of SAS (1999).

RESULTS

Serum biochemical indices of broiler chickens administered bovine testicular fluid at day 21

The serum biochemical parameters of broiler chickens administered graded levels of bovine testicular fluid at day 21 are presented in Table 1. There was no significant difference in the values obtained for the total protein, albumin, globulin, albumin/globulin ratio, aspartate aminotransferase, alanine amino transferase, alkaline phosphatase, creatinine, and cholesterol across the treatment groups.

Serum biochemical indices of broiler chickens administered bovine testicular fluid at day 49

The serum biochemical index of broiler chickens administered graded levels of bovine testicular fluid at day 49 is presented in Table 2. The value obtained for the total protein (TP) in the T5 (3.52 g/dL) was found to be significantly ($p < 0.05$) higher than the values obtained for the T4 (3.22 g/dL), T3 (3.14 g/dL), T2 (2.98 g/dL), and T1 (2.78 g/dL) across the treatment groups. The group of hens in T5 (32.80 iu/l) had the highest significantly ($p < 0.05$) value of alkaline phosphate (ALP) than T1 (24.80 iu/l), T2 (26.80 iu/l), T3 (27.00 iu/l) and T4 (27.20 iu/l) birds. Though there was no significant difference in the values obtained for the albumin, globulin, aspartate aminotransferase, the alanine amino transferase, creatinine, albumin/globulin ratio and the cholesterol among the treatments.

Haematological parameters of broiler chickens administered bovine testicular fluid at day 21

The effect of different concentrations of bovine testicular fluid on the haematological parameters of broiler chickens is presented in Table 4. There was no significant difference in the values obtained for the packed cell volume, white blood cell, red blood cell, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, lymphocytes, monocytes, and

eosinophils. The birds administered 75 ml of the bovine testicular fluid had the highest significant ($p > 0.05$) value of the haemoglobin 9.32 g/dL than the T1 (9.04 g/dL), T4 (9.00 g/dL), T1 (9.04 g/dL) and T5 (7.83 g/dL) respectively. The values obtained for the neutrophils show that birds administered with 75 ml of the bovine testicular fluid had the highest value of 34.30% which was significantly ($p < 0.05$) higher than T1 (31.00%), T2 (31.20%), T4 (30.40%) and T5 (30.29%) respectively. Likewise, the values obtained for the basophils count did not follow a regular pattern— similar to the reports of Abijo (2017). It was found that the value obtained for the T1 bird (4.20%) that was administered with 0 ml of the bovine testicular fluid was significantly higher ($p < 0.05$) than the other treatment group (T2 = 3.00%, T4 = 3.10%, T5 = 3.00% and T3 = 2.90%) respectively.

Haematological parameters of broiler chickens administered bovine testicular fluid at day 49

The effect of different levels of crude testosterone in bovine testicular fluid on the haematological parameters of broiler chickens is presented in Table 4. There was no significant difference in values obtained for packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, haemoglobin, lymphocyte, monocyte, eosinophil, neutrophils and basophils among the treatment groups. The values obtained for white blood cell shows that T4 ($16.01 \times 10^3/\text{mm}^3$) and T5 ($16.26 \times 10^3/\text{mm}^3$) was a significantly higher ($p < 0.05$) than T3 ($14.34 \times 10^3/\text{mm}^3$), T2 ($13.33 \times 10^3/\text{mm}^3$) and T1 ($15.61 \times 10^3/\text{mm}^3$). The values obtained for platelets shows that T1 ($27.56 \times 10^3/\text{mm}^3$) was significantly higher ($p < 0.05$) than T5 ($19.57 \times 10^3/\text{mm}^3$), T4 ($21.46 \times 10^3/\text{mm}^3$), T3 ($26.11 \times 10^3/\text{mm}^3$) and T2 ($25.24 \times 10^3/\text{mm}^3$) respectively, while the value obtained for the red blood cells also shows that the T5 (3.63 ± 0.03) and T4 (3.54 ± 0.05) had the highest significant ($p < 0.05$) values compared to T2 (3.36 ± 0.11), T3 (3.16 ± 0.024), and T1 (3.02 ± 0.23) respectively.

The intestinal mucosal assessment of broiler chickens administered different levels of bovine testicular fluid

The effect of the different concentration of bovine testicular fluid on the ileal mucosa development in broiler chickens is presented in Table 5. There were significant differences ($p < 0.05$) in the mean values obtained for the villus height across the treatments. The birds on T5 (4966.6 μm) and T4 (4952.6 μm) birds were significantly ($p < 0.05$) higher than the values obtained for the T3 (4343.3 μm), T2 (4294.2 μm), and T1 (4716.2 μm) birds, respectively. Significant differences ($p < 0.05$) was observed in the values obtained for the villi width as the values obtained ranged from 30.13 to 39.86 μm with T5 birds having higher

Table 1. Serum biochemical indices of broiler chickens administered bovine testicular fluid at day 21

Parameter	T1	T2	T3	T4	T5	P value
Total protein (g/dL)	2.58±0.13	2.68±0.12	2.70±0.20	3.08±0.22	2.70±0.11	0.28
Albumin (g/dL)	0.74±0.17	0.84±0.14	0.74±0.15	1.10±0.21	0.84±0.15	0.55
Globulin (g/dL)	1.94±0.08	2.04±0.17	1.96±0.07	1.98±0.09	2.00±0.05	0.96
A/G Ratio	0.36±0.09	0.36±0.04	0.34±0.04	0.54±0.12	0.42±0.07	0.44
AST (iu/l)	190.40±2.27	195.00±0.63	190.80±1.85	191.20±1.46	204.40±10.60	0.27
ALP (iu/l)	28.20±2.80	27.40±1.50	29.00±1.30	26.60±1.08	26.80±0.86	0.83
ALT (iu/l)	399.20±45.74	335.40±36.74	365.20±38.52	336.60±39.06	370.20±26.71	0.72
Creatinine (mg/dL)	0.58±0.04	0.66±0.04	0.58±0.05	0.68±0.06	0.58±0.04	0.34
Cholesterol (mg/dL)	156.00±12.16	197.00±24.58	203.60±20.85	205.20±24.58	211.80±17.25	0.30

Different treatments; T1= control (0 ml), T2 = 25 ml, T3 = 50 ml, T4 = 75 ml, T5 = 100 ml, AST= aspartate aminotransferase, ALP = alkaline phosphatase, ALT= alanine amino transferase, A/G ratio = Albumin/globulin ratio.

Table 2. Serum biochemical indices of broiler chickens at day 49.

Parameters	T1	T2	T3	T4	T5	SEM
Total protein (g/dl)	2.78±0.15 ^b	2.98±0.13 ^b	3.14±0.14 ^{ab}	3.22±0.14 ^{ab}	3.52±0.24 ^a	0.05
Albumin (g/dL)	0.88±0.15	0.940±0.17	1.180±0.18	1.34±0.13	1.40±0.18	0.13
Globulin (g/dL)	2.12±0.18	2.04±0.04	1.96±0.07	1.88±0.07	2.13±0.07	0.36
A/G Ratio	0.40±0.10 ^a	0.44±0.10 ^{ab}	0.58±0.11 ^{ab}	0.72±0.08 ^a	0.64±0.07 ^{ab}	0.13
AST (iu/l)	193.60±6.07 ^a	195.80±5.93 ^a	197.20±6.00 ^a	197.60±12.35 ^a	209.70±13.58 ^a	0.79
ALP (iu/l)	24.80±0.97 ^b	26.80±1.32 ^b	27.00±1.82 ^b	27.20±1.74 ^b	32.80±2.24 ^a	0.03
ALT (iu/l)	270.00±25.64 ^b	300.20±44.09 ^{ab}	306.60±24.29 ^{ab}	333.20±33.86 ^{ab}	384.00±26.84 ^a	0.17
Creatinine (mg/dL)	0.54±0.02	0.62±0.06	0.64±0.02	0.64±0.02 ^a	0.68±0.10	0.47
Cholesterol (mg/dL)	150.80±7.28	153.00±6.85	171.20±19.07	180.40±20.68	182.60±23.87	0.56

^{ab}=means on the same row with different superscripts are significantly ($p<0.05$) different. T1 = control (0 ml), T2 = 25 ml, T3 = 50 ml, T4 = 75 ml, T5 = 100 ml, AST= aspartate aminotransferase, ALP = alkaline phosphatase ALT=alanine amino transferase, A/G ratio = Albumin/globulin ratio.

Table 3. Haematological parameters of broiler chickens administered bovine testicular fluid at day 21.

Parameters	T1	T2	T3	T4	T5	SEM
PVC (%)	27.00±1.32	26.20±0.72	28.20±0.96	27.30±0.80	27.20±0.58	0.71
Hb (g/dL)	9.04±0.48 ^{ab}	8.70±0.26 ^{ab}	9.32±0.35 ^a	9.00±0.32 ^{ab}	7.83±0.24 ^b	0.89
RBC ($\times 10^6/\text{mm}^3$)	3.00±0.23	3.00±0.11	3.00±0.24	3.00±0.05	3.00±0.03	0.08
WBC ($\times 10^3/\text{mm}^3$)	16.19±367.64	20.22±1540.42	18.15±589.75	18.78±816.46	23.98±470.63	0.19
MCV (μ^3)	961.4±68.51	971.6±58.20	912.6±51.33	888.2±38.41	794.7±148.00	0.57
MCH (μg)	32.02±2.12	32.12±1.74	30.02±1.56	29.33±1.41	26.42±4.88	0.55
MCHC (%)	33.40±0.37	33.15±0.35	32.98±0.45	32.98±0.35	29.23±2.75	0.13
Platelet ($\times 10^3/\text{mm}^3$)	19.31±1.07	19.97±1.92	19.18±2.20	20.62±2.01	17.17±19.99	0.89
Lymphocytes (%)	61.60±1.59	62.40±1.85	60.40±2.17	62.80±2.03	63.10±1.47	0.43
Neutrophils (%)	31.00±1.67 ^{ab}	31.20±1.93 ^{ab}	33.30±2.26 ^a	30.40±1.64 ^{ab}	30.29±1.64 ^b	0.05
Monocytes (%)	3.90±0.33	3.20±0.20	3.10±0.27	3.30±0.31	3.40±0.23	0.78
Basophils (%)	4.20±0.47 ^a	3.00±0.50 ^{ab}	2.90±0.53 ^{ab}	3.10±0.57 ^{ab}	3.00±0.41 ^b	0.11
Eosinophils (%)	0.20±0.13	0.30±0.13	0.30±0.15	0.40±0.13	0.40±0.13	0.69

^{ab}=means on the same row with different superscripts are significantly ($p<0.05$) different. T1= control (0ml), T2 = 25 ml, T3 = 50 ml, T4=75ml, T5 = 100 ml, CTE = crude testosterone MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, PCV = packed cell volume, WBC = white blood cell, RBC = red blood cell, Hb = haemoglobin.

Table 4. Haematological parameters of broiler chickens administered bovine testicular fluid at day 49.

Parameters	T1	T2	T3	T4	T5	SEM
PCV (%)	29.50±1.32	30.10±0.72	31.60±0.96	31.70±0.81	31.60±0.58	0.29
Hb (g/dL)	9.42±0.48	9.65±0.26	9.70±0.35	10.33±0.32	10.05±0.24	0.36
RBC(x10 ⁶ /mm ³)	3.02±0.23 ^b	3.36±0.11 ^{ab}	3.16±0.0.24 ^{ab}	3.54±0.05 ^a	3.63±0.03 ^a	0.36
WBC (x10 ³ /mm ³)	15.61±3.67 ^{ba}	13.33±4.87 ^c	14.34±5.89 ^{cb}	16.01±8.16 ^a	16.26±4.70 ^a	0.00
MCV (μ ³)	1017.41±65.00	901.28±25.54	1001.88±87.88	896.78±21.58	869.78±15.29	0.17
MCH (μg)	32.57±0.49 ^a	32.04±0.26 ^{ab}	32.52±0.24 ^{ab}	32.57±0.43 ^{ab}	32.89±0.89 ^b	0.79
MCHC (%)	32.52±2.25	28.85±0.740.74	32.51±2.75	29.18±0.72	27.71±0.82	0.16
Platelet (x10 ³ /mm ³)	27.56±1.07 ^a	25.24±1.92 ^{ab}	26.11±2.01 ^{ab}	21.46±2.20 ^{ab}	19.57±1.99 ^c	0.02
Lymphocyte (%)	63.90±1.59	61.80±1.85	62.00±2.17	62.50±2.03	61.30±1.47	0.74
Neutrophils (%)	29.10±1.67	31.00±1.93	29.70±2.26	29.90±2.26	32.40±1.64	0.69
Basophils (%)	3.20±0.33	2.80±0.20	2.90±0.28	3.10±0.31	3.10±0.23	0.84
Eosinophils (%)	3.80±0.47	4.40±0.50	5.20±0.53	4.80±0.57	3.90±0.41	0.23
Monocyte (%)	0.20±0.13	0.20±0.13	0.30±0.15	0.20±0.13	0.20±0.13	0.98

^{ab}= means on the same row with different superscripts are significantly different (p<0.05). T1= (control) 0ml, T2 = 25 ml, T3 = 50 ml, T4=75 ml, T5 = 100 ml, CTE = crude testosterone MCV = mean corpuscular volume, MCH = mean corpuscular haemoglobin, MCHC = mean corpuscular haemoglobin concentration, PCV = packed cell volume, WBC = white blood cell, RBC = red blood cell, Hb = haemoglobin.

Table 5. Ileal mucosal development of broiler chickens administered different levels of Bovine testicular fluid.

Parameters	T1	T2	T3	T4	T5	SEM
Villi height (μm)	4716.2±118.85 ^{ab}	4294.2±122.93 ^b	4343.3±172.75 ^b	4952.6±151.34 ^a	4966.6±147.30 ^a	0.01
Villi width (μm)	415.43±16.92 ^c	497.36±28.57 ^b	473.07±26.16 ^{bc}	499.57±22.82 ^b	591.38±26.55 ^a	0.00
Villi density (μm)	31.39±1.24 ^b	34.07±1.59 ^b	30.13±1.11 ^b	30.54±1.04 ^b	39.86±1.43 ^a	0.00
Cryptal depth(μm)	2343.5±217.35	2564.6±91.87	2483.9±108.42	2244.4±85.12	2463.6±94.48	0.44
Cryptal width (μm)	412.30±21.19	415.21±20.61	369.72±22.22	350.58±20.70	359.17±20.79	0.08
Mucosal thickness (μm)	7517.3±147.69 ^a	7179.8±132.04 ^a	7450.6±191.58 ^a	6637.7±236.52 ^b	6587.6±226.92 ^b	0.01
Intestine weight (g)	12.13±1.30 ^c	15.59±0.62 ^b	13.69±0.68 ^{bc}	14.15±12.56 ^{bc}	17.98±0.74 ^a	0.00
Intestine length (cm)	256.14±0.69 ^a	256.38±7.632 ^b	264.13±4.80 ^b	262.63±2.30 ^b	282±5.37 ^a	0.01

^{abc}= means on the same row with different superscripts are significantly (p<0.05) different. T1= control (0ml), T2 = 25 ml, T3 = 50 ml, T4 =75 ml, T5 =100

significant values of 39.86 μm than the T1(415.43 μm). The villi density also ranged from 30.13 to 39.86 μm. This was significantly higher (p<0.05) in T5 (39.86 μm) than the T1 birds (31.39 μm). There was no significant difference in the values obtained for the cryptal depth and the cryptal width among the treatments. There was no significant difference (p>0.05) in the values obtained for the mucosa thickness, but it was observed that the value obtained for the T1 (7517.3 μm), T2 (7179.8 μm), and T3 (7450.6 μm) were higher than that of the T4 (6637.7 μm) and T5 (6587.6 μm). There was a significant difference (p<0.05) in the values obtained from the intestinal weight, with T5 having the highest value (17.98 g) and T1 (12.13 g) having the least value. There was a significant difference (p<0.05) in the intestinal length, with T5 having the highest value (282 cm) followed by T1 (256.14 cm), T3 (264.13 cm), T4 (262.63 cm), T2 (256.38 cm).

DISCUSSION

Serum biochemical and haematological parameters are

reliable indicators of the health status of animals, and may have important roles in diagnosis, prognosis, as indicators, and treatments of poultry diseases (Olafedehan et al., 2010). Factors affecting serum biochemical parameters are nutrition, season, climate change, sex, age, type of bird, and management system (Bell, 1971; Forlan et al., 1999).

The total protein levels across the treatments increased gradually up to birds that received 75 ml/liter of the bovine testicular fluid, though the observed total protein and albumin were lower than the values reported by Mitruka and Rawnsley (1977). This could be an indication of hypoalbuminemia (Altman, 1979), which corroborates the report of Bowes et al. (1989) who documented that albumin level will not increase when the protein intake did not exceed the amount required for growth and maintenance. The birds were 21 days old, with one week for their adaptation period, and, as such, they need more protein for growth and maintenance. According to Bell (1971), total protein is influenced by breed, age, environment, physiological state, and sex. The results of

the total protein and albumin obtained from this study is consistent with the findings of Mitruka and Rawnsley (1981) and Ross et al. (1976), who stated that the total protein and albumin level is lower in younger birds.

The ALT, AST, and the ALP values of the treated birds were found within the normal range given by Mitruka and Rawnsley (1977). Though a slight increase in the value of AST was observed which follows the inclusion level of the bovine testicular fluid of the treated birds, with T5 (100 ml/L of the crude testosterone) birds having the highest expression of the liver enzyme (204.40 iu/l). The absence of abnormal rise of AST in the bloodstream of treated birds suggests that the bovine testicular fluid administered to the birds did not cause liver or other organ damage. The Alkaline phosphatase fell within the range of Mitruka and Rawnsley (1977), though the expression of this liver enzyme did not follow a regular pattern. The normal value of these liver enzymes may indicate that the organs of the treated birds were not stressed or damaged and the inclusion of the bovine testicular fluid in the drinking water of the treated birds did not have any adverse effect on the health status of the birds. Besides, the expression of the creatinine enzyme in the serum of the treated birds further indicated there were no abnormal changes in the serum levels of creatinine kinase (CK) or creatinine phosphate kinase (CPK) in the kidney, liver and heart of the treated birds.

The cholesterol values obtained was above the upper limit of the reference range given by Mitruka and Rawnsley (1977). Although there was no significant difference between the treated group and the control which suggests that the inclusion of the testicular fluid in the drinking water of the birds did not have any negative effect on the lipid profile or cholesterol level of the treated birds as it competes favourably with the control (Matsumoto, 2002).

The serum biochemical parameters at day 49 revealed no significant difference among the treatment groups except for the alkaline phosphate and total protein. The activities of the liver enzyme are relatively low, but increases rapidly in the blood following muscle or liver damage. However, the increase in the total protein is an indication of proper utilization of dietary protein possibly stimulated by crude testosterone in the testicular fluid.

Testosterone or one of its physiologically active metabolites binds to receptors in muscle and stimulates increased incorporation of amino acids into protein, which also agrees with the findings of Ross et al. (1979), who found that the total protein and globulin increased for the first eight weeks in leghorn chickens. Though the values obtained were still below the recommended range reported by Mitruka and Rawnsley (1977) and Wikivet (2012), but the results obtained suggest that the inclusion of bovine testicular fluid tends to increase the expression of the total protein in serum of the treated birds than the control birds, which may probably help in the production of antibody to fight diseases and infections.

The AST and ALP that were within the reference range of Mitruka and Rawnsley (1977) suggest that the increase

in the testicular fluid that contained crude testosterone did not affect the specific and non-specific liver enzyme. The AST and ALT values obtained in this present research for birds at day 49 were within the recommended range by Mitruka and Rawnsley (1977) except the AST of the birds administered 100 ml/L which exceeded the upper limit. This suggests that higher bovine testicular fluid may have possibly impaired the kidney and/or liver (Nishino, 1975; Hadelsman and Gupta, 1997; Filova et al., 2013).

There was no significant difference in the values obtained for the creatinine across the treatment group. However, as the bovine testicular fluid increased across the treatments, there was a slight increase in the creatinine levels, although the values obtained were low compared to the report of Mitruka and Rawnsley (1977) and WikiVet (2012). The low creatinine values could also suggest that there was a decrease in muscle metabolism. Low muscle mass is associated with low creatinine levels (Sissons, 2017). The cholesterol levels obtained were found to increase with the inclusion levels of the bovine testicular fluid. The level in the birds administered 0 ml/L and 25 ml/L were within the recommended range, but those that received 50, 75 and 100 ml/L testicular fluid, respectively, were above the range reported by Mitruka and Rawnsley (1977), which implies that there is a tendency the crude testosterone in the testicular fluid possibly precipitate an increase in cholesterol.

The haematological parameters examination is among the methods which may contribute to the detection of some changes in health and physiological status, which may not be apparent during physical examination, but which affects the fitness of the animal (Bamishaiye et al., 2009). On day 21, the bovine testicular fluid did not affect the health status of the treated since they compare favourably with the control birds. The values obtained for most of the haematological variables were within the recommended physiological range by Mitruka and Rawnsley (1977), which suggest that the bovine testicular fluid did not have any side effect on all the haematological parameters of the birds probably the birds were still acclimatizing to the test ingredient. Conversely, it was observed that there was a slight increase in the haematological parameters of the birds at 49 days of age. This suggested that the bovine testicular fluid did not have any adverse effect on the blood level of the treated birds neither did they suffer from anaemia. Though the result obtained were not significantly different among the treatments, but agreed with the report of Zhao et al. (2013) who found that testosterone showed a tendency to increase HB, PCV level at 6 weeks, and lowered blood viscosity in rabbits.

The addition of natural growth promoters to feeds or water of poultry or farm animals may have several beneficial effects, including the rapid development of a healthy gut microflora stabilization of digestion, increased growth performance, stimulation and rapid maturation of the immune system, reduced incidence of diarrhea, improved feed efficiency, lower mortality rates (Pasteiner, 2006). It is assumed that an increase in villus height is

paralleled by an increase in digestive and absorptive function of the intestine due to an increase of surface area, expressive of brush border enzymes and nutritional transport system (Pluske et al., 1996). Larger villus height is an indicator of intestinal villi activity (Langhout et al., 1999; Shamoto and Yamauchi, 2000). Long villi are usually associated with excellent gut health, increased absorptive efficiency and healthier GIT (Alfaro et al., 2007). According to Cera et al. (1988), well-developed enterocytes and longer villus height with a large capacity of the luminal area provide maximum absorption and digestion. According to the report of Ravindran et al. (1984) and Apajalahti et al. (2004), the gut microflora can reduce the nutrient uptake of the host animal by increasing the thickness of the gastro intestinal tract and also caused an increase in the rate of digesta passage. This will in turn increase the rate of turn-over of the gut mucosa by paving way for the gut microflora to compete with the host for a portion of dietary energy and protein released from the feed. It was observed that the birds had the highest intestinal weight and length compared to other treatments group, which corroborates the report of intake of exogenous feed is accompanied by the rapid development of the GIT and associated organs. It was observed that the treated birds grow rapidly than the control birds (Ewuola and Olujimi, 2019) due to the development of the ileum absorptive cell possibly induced by the bovine testicular fluid. In addition, the development of the intestinal mucosa of the birds was found to follow the trend of the increase in the inclusion level of the bovine testicular fluid administered to the birds. Birds administered 100 ml of bovine testicular fluid/liter of water was found to have the highest intestinal mucosal development which enhanced the rate of nutrient absorption and growth performance than the control and other treatment groups (Ewuola and Olujimi, 2019). The report of this study agreed with the finding of Miles et al. (2006) who reported that a well-developed intestinal morphology will improve various nutrient absorption and thereby enhance growth performance. Evidence from the report of Ulshen et al. (1993) suggests that bovine growth hormone could facilitate hyperplasia in the intestinal mucosa without depending on whether the feeding is *ad libitum* or restricted. This established the fact that the treated birds developed better intestinal mucosa compared to the control birds (though the treated birds and the control birds were fed *ad libitum*) as a result of the inclusion of the bovine testicular fluid which facilitated the development of the mucosa. This implies that the increased growth performance of the treated birds (Ewuola and Olujimi, 2019) was enhanced by the synergistic action of the androgen hormones and the nutrient released from the feed supplied to the birds which corroborates with the report of Ranaweera (1977) that in male and female turkeys, androgens hormones were found to increase the growth rate as well as feed conversion efficiency, possibly by enhancing the anabolic effect of the bovine testicular fluid.

Conclusion

The result of this study showed that bovine testicular fluid which can be easily obtained as a waste product from the abattoir can be used to enhance the feed nutrient absorptive capacity of the intestinal mucosa surface area and utilization thereby improve the growth performance of broiler chickens, the bovine testicular fluid did not have a deleterious effect on the haematological parameters as the immunity of the bird was not comprised. However, levels above 75 ml/L induce specific and non-specific enzymes and should be further investigated

Recommendations

From the result of the present study, it can be recommended that:

1. Up to 75 ml of the bovine testicular fluid extract can be diluted in one litre of water to improve the feed nutrient digestibility and absorption level of broiler chicken and therefore enhance its growth performance.
2. Further research on levels above 100 ml per litre of drinking water is recommended.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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