



SEMEN QUALITY, TESTICULAR MORPHOMETRY AND IMMUNE TRAITS OF NOILER COCKS GIVEN TAMARIND LEAVES AQUEOUS EXTRACT (*TAMARINDUS INDICA* L.) IN A HOT DRY ENVIRONMENT^[1]

Saleh, B.¹, Mahmoud, A. A.¹, Mohammed, A.¹, Adamu, J.¹, Haladu, I.¹, Jibrin, T.¹ and Zaro, H.²

¹Department of Animal Science, University of Maiduguri, Borno State, Nigeria

²Adamawa State Post Primary School Board, Yola Adamawa State, Nigeria

ABSTRACT

This study was designed to determine the effect of tamarind leaves aqueous extract on semen quality, testicular morphometry and immune traits of Noiler cocks in a hot dry environment. Twenty-four (24) Noiler cocks of thirty-two (32) weeks of age were used for the experiment. The birds were reared on deep litter and were fed *ad libitum* with a commercial feed (14% CP) throughout the twelve weeks' experimental period. The tamarind leaves aqueous extract (TLE), were obtained by boiling a known quantity of the leaves with tap water for ten minutes and allowed to cool and then filtered. The filtrate (extract) was given to the cocks as drinking water throughout the experimental period. Fresh extracts were prepared every day. The cocks were divided into four treatment groups, each replicated three times in a Completely Randomized Design and offered the tamarind leaves extract as follows; T1 = Control (No tamarind leaves extract), T2 = 5g tamarind leaves in 1litre of water, T3 = 10g tamarind leaves in 1litre of water, T4 = 15g tamarind leaves in 1litre of water. Data was collected on semen volume, colour, motility, concentration, testicular morphometry. Semen volume (0.43 ml) was highest ($p < 0.05$) in cocks given 10g TLE/l. Aqueous tamarind leaves extract had no effect on pH, mass motility and percentage abnormal sperm cells. Individual motility was low ($p < 0.05$) at 15gTLE/l. Sperm concentration and percentage dead sperm were highest ($p < 0.05$) in the group given 10g/l TLE. Cocks given 10g/l TLE had heavier ($p < 0.05$) left and paired testicular weights. There was significant influence of TLE on eosinophils, total protein, albumin and globulin, while means for white blood cells, neutrophils and lymphocytes were similar for all treatments. It was concluded that 10g/L aqueous tamarind leaves extract (TLE) positively influenced semen quality, testicular morphometry and immune response of Noiler cocks in a hot dry environment.

Keywords: Tamarind leaves, Semen quality, Testis weight, White blood cells, Total protein

INTRODUCTION

Flock infertility is a major factor that poultry breeders struggle with and approximately 50% of the problem is male related (Saleh *et al.*, 2017). The semen quality of the cock is a major determinant of its fertility. However, in semi-arid regions of the tropics where most poultry are reared under high environmental temperature in open sided pens, the maintenance of fertility in breeder cocks has

been difficult. In these tropical regions, high environmental temperature (heat stress) among other factors adversely affects semen production capacity of cocks (Ayo *et al.*, 2011). Animal's body responds to heat shock discomfort by overproduction of reactive oxygen species (ROS) which induces oxidative stress is another factor that can affect semen quality (Ngoula, *et al.*, 2020). Cock spermatozoa is known to have high

polyunsaturated fatty acids (Khan *et al.*, 2011). This high level of polyunsaturated fatty acids however, predisposes the spermatozoa to oxidative damage and associated spermatozoa dysfunction. Therefore, lipid peroxidation constitutes a major cause of infertility in males (Zaniboni *et al.*, 2006) and its effects can be ameliorated by the antioxidant system.

Plant polyphenols have been reported to have high antioxidant properties hence attention of scientist has been turned to them as sources of natural antioxidants (Hano and Tungmunnithum, 2020, Akraim *et al.*, 2022). Swelum *et al.* (2021) noted that there has been a growing interest in the use of phytochemicals to enhance the reproductive efficiency of livestock because of their antioxidant and anti-inflammatory properties and lower toxicity than synthetic antioxidants (Ezzat *et al.*, 2017; Saleh *et al.*, 2021).

Tamarind (*Tamarindus indica*) is a leguminous tree bearing edible fruits that is indigenous to tropical Africa (El-Siddig *et al.*, 2006). According to Razali *et al.* (2012), the seed, leaf, leaf veins, fruit pulp and skin extracts of tamarind possessed high phenolic content and antioxidant activities. Deepak *et al.* (2016), reported that tamarind leaves powder contains 4.65% moisture, 4.08 g crude protein, 1.52 g fat, 1.0 g crude fibre, 86.26 g carbohydrate, 375 Kcal energy and 2.5 g Ash/100 g. They further noted that vitamins like beta-carotene and vitamin C content were 166.8 µg and 2.4 mg/100 g while minerals such as iron and calcium were 0.18 mg and 96.4/100 g respectively. Rai *et al.* (2018), reported that aqueous extract of *Tamarindus indica* possessed aphrodisiac activity together with spermatogenic potential.

This study was designed to determine the effect of aqueous extract of tamarind leaves on testicular morphometry and semen quality of noiler cocks in a hot dry environment.

MATERIALS AND METHODS

This study was conducted at the Poultry Unit of the Teaching and Research Farm, Department of Animal science, Faculty of Agriculture, University of Maiduguri, Borno State, Nigeria. The area falls within the semi-arid zone, lies between latitude 11°05 and 12° north, and longitude 13 ° 05 and 14 ° east. It is characterized by short rain fall (3-4 months) which varies from 500mm, with long dry cold season (8-9 months). The ambient temperature could be as low as 20 ° c during the dry season (February-may). Relative humidity is about 5% in April and May. Day length varies from 11-12 months. (Kwari *et al.*, 2011).

The experiment was conducted between the months of March to June. The high and low ambient temperatures of the poultry house were recorded daily using a thermometer that records the maximum and minimum temperature for the day. To measure the thermal comfort of the experimental birds, daily average dry bulb temperature and Relative Humidity were recorded and used to calculate the Temperature Humidity Index (THI) using the expression of Marai *et al.* (2001):

$$THI = Tdb - \{(0.31 - 0.31 RH) (Tdb - 14.4)\}$$

Where;

THI is the temperature humidity index,

Tdb is the dry bulb temperature (°C) and

RH is the relative humidity (%)/100.

Experimental Stocks and Management

Twenty-four (24) Noiler cocks of thirty-two (32) weeks old were used for the experiment. The birds were reared on deep litter and were fed *ad libitum* with a commercial feed (14% CP) throughout the twelve weeks' experimental period.

Test Material

Fresh tamarind leaves were obtained from tamarind trees within the University of

Maiduguri by removing them from the stalk. Samples were identified at the herbarium of the Department of Forestry and Wild Life in the same University. The leaves were shade dried for 48 hours and then stored in airtight bags until used.

To obtain the aqueous leave extract, a known quantity of the leaves was weighed and boiled in tap water for ten minutes and allowed to cool and then filtered. The filtrate (extract) was given to the cocks as drinking water throughout the experimental period. Fresh extracts were prepared every day.

Experimental Design

The cocks were divided into four treatment groups, each replicated three times in a Completely Randomized Design and offered the tamarind leaves extract as follows;

T1 = Control (No tamarind leaves extract),

T2 = 5g tamarind leaves in 1litre of water

T3 = 10g tamarind leaves in 1litre of water

T4 = 15g tamarind leaves in 1litre of water

Data Collection

Semen samples were collected weekly by abdominal massage technique (Donoghue and Wishart, 2000) into pre-weighed 2ml sterile sample bottles. Semen volume was obtained as the difference between weight of sample bottle plus sample less the weight of empty bottles, assuming the density of semen to be 1 g/ml as recommended by the World Health Organization [WHO] (2010). Semen colour was visually assessed immediately after collection and scored as; 1 = creamy, 2 = creamy-white and 3 = opaque. A drop of semen was evenly placed on a pH paper (range 1 – 14), after the colour of the impregnated zone became uniform (< 30 seconds) it was compared with the calibrated strip to determine the pH (Saleh *et al.*, 2018). Mass motility was determined by placing a drop of raw undiluted semen on a prewarmed slide and covered with

a slip and viewed under microscope at 100x. Mass motility was estimated as by wave pattern as follows; 4 – rapid swirling, 3 – Slower swirling, 2 – generalized oscillation and 1 – sporadic oscillation. Individual sperm motility was examined at a magnification of 400x. Several fields were examined and an estimate to the nearest 10% of motile sperm was made. The motility determination was carried out by taking into consideration subjective measurements and it was expressed as the percentage of motile sperm with moderate to rapid progressive movement (Saleh *et al.*, 2018). Percentage normal and dead spermatozoa were evaluated using the eosin/nigrosin staining procedure (WHO, 2010).

Semen concentration was determined with the improved Neubauer haemocytometer using the direct cell count method (WHO, 2010). The concentration of sperm per volume was determined using the method of Peters *et al.* (2008):

$$C = 50,000 \times N \times D$$

Where C = Concentration of semen per volume (ml),

N = Number of spermatozoa counted,

D = Dilution rate.

At the end of the experiment, paired testes were collected from each cock, trimmed of all adhering fat and tissues and weighed to the nearest 0.01g using an electronic scale. Testis length and width were measured using a digital Vernier calliper to the nearest 0.01 mm. Volume was obtained using Archimedes principle (Saleh *et al.* 2017). Gonadosomatic index was obtained using the following expression;

$$GSI = \frac{\text{Testicular weight}(g)}{\text{Live weight}(g)} \times 100$$

While Testis density was calculated as follows;

$$\text{Testis Density} = \frac{\text{Testicular weight}}{\text{Testicular volume}}$$

Statistical analysis

Data were subjected to a one-way Analysis of Variance (ANOVA) using the General linear model of the Statistix version 9.0 software. Significant means, were separated using the least significant difference.

RESULTS AND DISCUSSION

Average ambient temperatures were 30.5, 39.3, 35.1 and 32.5 °C (high) and 23.6, 23.7, 22.2 and 21.5 °C (low) for March, April, May and June respectively. Fernandes *et al.* (2023) noted that the thermoneutral zone for laying hens was 19–22 °C and 18–22 °C for broilers. This shows that the birds reared in this experiment were above their zone of thermal comfort. The average THI for March, April, May and June were 25.99, 29.33, 29.90 and 28.34 respectively. According to Marai *et al.* (2001), THI based stress ranges for poultry are as follows: normal <27.8, moderate 27.8–28.8, severe 28.9–29.9 and very severe (emergency) ≥30.0. which shows that the birds were under heat stress. The supplementation of natural antioxidants is essential to relief the adverse effects of heat stress (El-Ratel *et al.*, 2021).

The effects of aqueous extract of tamarind leave meal on semen quality of Noiler cocks in a hot dry environment are shown on Table 1. Semen volume was highest (p<0.05) in cocks

given 10g TLE/l and the control while those given 5 and 15g TLE/l had lower means. Rai *et al.* (2018) had earlier reported that tamarind increased sperm production in male Wistar rats. Similarly, Saleh *et al.* (2021) reported increase in semen volume when Noiler cocks were offered an aqueous extract of tamarind pulp. The increased semen volume is an indication of spermatogenic potentials of tamarind probably due to its antioxidant properties (Deepak *et al.*, 2016). Aqueous tamarind leaves extract had no effect on Ph, mass motility and percentage abnormal sperm cells. Individual motility was low (p<0.05) at 15TLE/l. Sperm concentration and percentage dead sperm were highest (p<0.05) in the group given 10g/l TLE. Concentration per ejaculate, and total motile sperm were similar for control and 10g/l TLE while cocks given 5 and 15g/l TLE had lower (p<0.05) means than the control. Results of this study show that 10g/l TLE level seems to be the threshold amount that would enhance semen quality of Noiler cocks. Beyond 10g/l TLE level, the diet appeared to have a negative impact on the quality of semen. The results recorded in this experiment for semen volume, mass activity and sperm motility were similar to those reported by Saleh *et al.* (2021) for volume (0.22 – 0.42ml), mass motility (3.14 - 4.39) and sperm motility (64.78 – 87.11%) respectively when Noiler cocks were given aqueous tamarind pulp extract. Semen colour and Ph were similar to those reported by Amur *et al.* (2020) for different strains of Noiler cocks.

Table 1: Effect of aqueous extract of tamarind leave meal on semen quality of Noiler cocks in a hot dry environment

Parameters	Treatments				SEM
	0g TLE	5g TLE	10g TLE	15g TLE	
Volume (ml)	0.38 ^a	0.26 ^b	0.43 ^a	0.23 ^b	0.05*
pH	6.89	7.00	6.92	7.00	0.09 ^{NS}
Colour	Creamy	Creamy	Creamy	Creamy	NA
Mass Motility	3.67	4.00	3.92	3.58	0.22 ^{NS}
Motility (%)	83.33 ^a	83.33 ^a	82.31 ^a	74.17 ^b	4.29*
Concentration (X 10 ⁹)	3.52 ^b	3.58 ^b	4.35 ^a	3.40 ^b	0.26*
Concentration/ejaculate (X 10 ⁹)	1.58 ^a	0.78 ^b	1.66 ^a	0.77 ^b	0.22*
Total Live sperm/ml (x10 ⁹)	3.50 ^{ab}	3.32 ^b	4.20 ^a	3.42 ^b	0.25*
Total motile sperm/ml (x10 ⁹)	2.90 ^{ab}	2.76 ^b	3.55 ^a	2.61 ^b	0.27*
Abnormal sperm (%)	2.26	1.71	1.43	0.87	0.74 ^{NS}
Dead sperm (%)	2.01 ^a	0.28 ^b	0.72 ^b	0.85 ^b	0.52*

TLE – Tamarind leave extract, SEM – Standard error of mean, NS – Non Significant, ^{a,b,c,...}Means in same row bearing different superscript differ significantly (p<0.05)

Table 2 shows the effect of aqueous extract of tamarind leave on testicular morphometry of Noiler cocks in a hot dry environment. Cocks live weights were similar among all the treatment groups. Right testes weight was not influenced by the treatment. Cocks given 10g/l TLE had heavier (p<0.05) left and paired testicular weights than those give 5g/l TLE. This may account for the higher semen volume by cocks given 10g/l TLE. Left testis were longer (p<0.05) for cocks given 15g/l TLE while right testis length was similar among all the treatments. Right and left testis width were lowest (p<0.05) for cocks given 5g/l TLE while those given 10g/l TLE had the highest. The control group had the highest (p<0.05) testicular volume while those offered 5g/l TLE had the lowest means. Testicular density and gonadosomatic index were greater (p<0.05) for cocks offered 10g/l TLE than those in the control group.

Haematological and serum biochemical responses are used to assess the clinical and

physiological responsiveness and well-being of chickens. Abnormalities in the values of these substance may indicate presence of disease or some other physiological disorders. Table 3 show the effect of aqueous tamarind leaf extract on immune response of Noiler Cocks in a hot dry environment. There was significant influence (p<0.05) of TLE on eosinophils, total protein, albumin and globulin, while means for white blood cells, neutrophils and lymphocytes were similar for all treatments. White blood cells (WBC) and its derivatives are good indicators of the immune response of a chicken. Leukocytes play important role in removing invading antigens (e.g. Bacteria) and to some extent transport and distribute antibodies. Eosinophils are found especially in areas where there is an allergic reaction (Lombardi et al., 2022). The absence of eosinophils in the groups given TLE at 5 and 10% may be an indication that it suppressed whatever caused allergic reactions in the control and 15% TLE group.

Table 2: Effect of aqueous extract of tamarind leave on testicular morphometry of Noiler cocks in a hot dry environment

Parameters	Treatments				SEM
	0g TLE	5g TLE	10g TLE	15g TLE	
Live weight (g)	3033	3033	3033	3167	179.51 ^{NS}
Right testis weight (g)	12.61	12.56	18.61	15.39	3.14 ^{NS}
Left testis weight (g)	12.52 ^{ab}	10.79 ^b	17.18 ^a	13.80 ^{ab}	2.74*
Paired testes weight (g)	25.11 ^{ab}	23.33 ^b	35.74 ^a	29.10 ^{ab}	5.58*
Right testis length (mm)	40.13	43.37	47.00	45.33	3.43 ^{NS}
Left testis length (mm)	40.17 ^b	40.30 ^b	43.33 ^b	48.33 ^a	2.06*
Right testis width (mm)	22.73 ^{ab}	19.70 ^b	25.00 ^a	20.33 ^{ab}	2.50*
Left testis width (mm)	22.13 ^{ab}	20.33 ^b	26.00 ^a	19.47 ^b	2.31*
Right testis volume (ml)	25.70 ^a	12.67 ^b	15.00 ^{bc}	21.67 ^{ab}	4.28*
Left testis volume (ml)	25.37 ^a	13.33 ^b	18.67 ^{ab}	19.00 ^{ab}	4.12*
Right epididymis weight (g)	0.37	0.28	0.33	0.37	0.07 ^{NS}
Left epididymis weight (g)	0.38 ^a	0.19 ^b	0.34 ^a	0.38 ^a	0.04*
Paired Testis Density (g/ml)	1.19 ^b	1.76 ^{ab}	1.92 ^a	1.75 ^{ab}	0.34*
Gonadosomatic index (%)	0.82 ^b	0.77 ^b	1.18 ^a	0.90 ^{ab}	0.16*

TLE – Tamarind leave extract, SEM – Standard error of mean, NS – Non Significant
^{a,b,c,...}Means in same row bearing different superscript differ significantly (p<0.05)

Table 3: Effect of aqueous tamarind leaf extract on immune response of Noiler Cocks in a hot dry environment

Parameters	T1	T2	T3	T4	SEM
	(control)	(5g/l TLE)	(10g/l TLE)	(15g/l TLE)	
WBC (x10 ³ /mm ³)	10.93	10.77	10.40	10.87	0.34 ^{NS}
Neutrophil (%)	23.33	31.33	24.67	25.67	4.24 ^{NS}
Eosinophil (%)	1.67 ^a	0.00 ^b	0.00 ^b	2.33 ^a	0.76*
Lymphocytes (%)	75.00	68.67	75.33	71.67	4.43 ^{NS}
Total Protein (g/dl)	7.00 ^a	4.73 ^b	6.00 ^a	5.90 ^{ab}	0.59*
Globulin (g/dl)	2.63 ^a	1.70 ^b	2.23 ^{ab}	2.03 ^{ab}	0.37*
Albumin (g/dl)	4.37 ^a	3.03 ^c	3.77 ^b	3.87 ^{ab}	0.26*

^{a, b, c, ...} means within the same row bearing different superscript differ significantly (P < 0.05). SEM= Standard error of mean, TLE – Tamarind leaves extract

Serum proteins are mainly synthesized in the liver, and, among other functions participate in the body defense against foreign agents (Rezende *et al.*, 2017). Total protein and globulin levels were lower in cocks offered 5g/L TLE compared to the control group. Albumin was lowest in the group offered 5 g/L TLE followed by those on 10g/L TLE. According Meluzzi *et al.* (1992) the normal ranges of the TP and ALB in bird's blood are 3.0-4.9 mg/dl and 1.17-2.74 g/dl, respectively.

Similarly, Board *et al.* (2018), reported that serum total protein and albumin values of backyard chickens ranged from 3.6 to 7.1 and 1.5 to 3.3 g/L respectively. Similarly, Joseph *et al.* (2021) reported total protein range of 4.1-4.4 mg/dl for noiler cocks offered aqueous tamarind pulp extract. Globulin was lowest for the cocks offered 5 g/L TLE although it was similar to the other groups given TLE. The control group however had numerically higher Globulin mean. Globulin a heterogenous group

of large serum proteins plays an important role in liver function and fighting against infections. Increased levels of globulins are often associated with infectious diseases, immune-mediated disease and oxidative stress. A higher concentration of albumin usually denotes dehydration while a lower concentration may be due to the liver not functioning adequately due to factors such as malnutrition and infection (Odunitan-Wayas *et al.*, 2018).

REFERENCES

- Akraim, F., Alfakhri, M. Y. M. and Bellail, A. A. (2022). The effect of rosemary (*salvia rosmarinus*) supplemented diet on reproductive and productive traits of Libyan local pigeon. *Slovak J. Anim. Sci.*, 55 (1–4): 47–54.
- Amur, S., Kalla, D. J. U. and Mancha, Y. P. (2020) Semen characteristics and fertility of three strains of Noiler cocks. In: The Book of Proceedings of the 45th Annual Conference of the Nigerian Society for Animal Production, Bauchi, Bauchi State, Nigeria. March 16 – 19, 2020. Pp. 234 – 237.
- Ayo, J. O., Obidi, I. J. A. and P. I. Rekwot. (2011). Effects of heat stress on the wellbeing, fertility, and hatchability of chickens in the northern guinea savannah zone of Nigeria: a review. *ISRN Vet. Sci.*, Doi:10.5402/2011/838606
- Board, M. M., Crespo, R., Shah, D. H. and Faux, C. M. (2018). Biochemical Reference Intervals for Backyard Hens. *J. Avian Med. and Surg.*, 32(4):301–306.
- Deepak, U. S. H., Yadav, D. K. K. and Suguna, M. (2016). Nutrient Composition of Dehydrated Tender Tamarind Leaves (*Tamarinds indicia L.*) Powder. *Indian J. Sci. Technol.*, 9(37) DOI: 10.17485/ijst/2016/v9i37/100005
- Donoghue, A. M. and Wishart, G. J. (2000). Storage of poultry semen. *Anim. Repro. Sci.*, 62: 213-232.
- El-Ratel, I. T., Attia, K. A. H., El-Raghi, A. A. and Fouda, S. F. (2021). Relief of the negative effects of heat stress on semen quality, reproductive efficiency and oxidative capacity of rabbit bucks using different natural antioxidants. *Anim. Biosci.*, 34 (5): 844-854. <https://doi.org/10.5713/ajas.20.0258>
- El-Siddig, K., Gunasena, H. P., Prasad, B. A., Pushpakumara, D.K., Ramana, K.V., and Viyayanand, P. *et al.* (2006). In: Williams, J.T., Smith, R.W., Haq, N., and Dunsiger, Z. (editors). *Tamarindus indica Fruits for the Future*. 1st ed. Southampton centre for underutilized crop RPM print and design, W. sussex, England, page 188.
- Ezzat, W., El-Slamony, A. E., Bealish, A. M. A., Ouda, M. M. M. and Sabry, M. M. (2017). Effect of adding dried ginger rhizomes to diets on semen quality and fertility rate in aged local cocks under Egyptian hot summer condition. *Egyptian Poult. Sci. J.*, 37(1): 233-249.
- Fernandes, E., Raymundo, A., Martins, L.L., Lordelo, M. and de Almeida, A.M. (2023). The Naked Neck Gene in the Domestic Chicken: A Genetic Strategy to Mitigate the Impact of Heat Stress in Poultry Production—A Review. *Animals*,

CONCLUSION

The use of aqueous tamarind leaves extract (TLE) is shown to positively influence semen quality, testicular morphometry and immune response of Noiler cocks in a hot dry environment. It can therefore be recommended that Noiler cocks reared under hot dry environment be given 10g/L of aqueous TLE to improve semen quality and immunity.

- 13, 1007. <https://doi.org/10.3390/ani13061007>
- Hano, C. and Tungmunnithum, D. (2020). Plant polyphenols, more than just simple natural antioxidants: oxidative stress, aging and age related diseases. *Medicines*, 7: 26; doi:10.3390/medicines7050026
- Joseph, J. N., Adamu, J., Mahmoud, A. A., Wachap, A. and Saleh, B. (2021). Effect of aqueous tamarind pulp extract on haematological parameters and serum biochemical indices of Noiler cocks. In: Proceedings of 26th Annual conference of ASAN-NIAS, Uyo, Nigeria.
- Khan, R. U., Nikousefat, Z., Javdani, M., Tufarelli, V. and Laudadio, V. (2011). Zinc induced moulting: production and physiology. *Worlds Poult. Sci. J.*, 67:497–506.
- Kwari, I. D., Saleh, B., Diarra, S. S., Mkighir, T. and Umanah, M.J. (2011). Nutrient digestibility carcass characteristics of broiler chickens fed different cultivars of sorghum replacing maize in the semi-arid zone of Nigeria. *ROAVS*, 1(9): 578- 581.
- Lonbardi, C., Berti, A. and Cottini, M. (2022). The emerging roles of eosinophils: Implications for the targeted treatment of eosinophilic-associated inflammatory conditions. *Curr. Res. in Immunol.*, 3: 42-53. <https://doi.org/10.1016/j.crimmu.2022.03.002>.
- Marai, I. F. M., Ayyat, M. S. and Abd El-Monem, U.M. (2001). Growth performance and reproductive traits at first parity of New Zealand White female rabbits as affected by heat stress and its alleviation under Egyptian conditions. *Trop. Anim. Health. Prod.*, 33(6): 457-462.
- Meluzzi, A., Primiceri, G., Giordani, R. and Fabris, G. (1992). Determination of blood constituents reference values in broilers. *Poult. Sci.*, 71(2):337-345.
- Ngoula, F., Lontio F. A., Tchoffo, H., Manfo Tsague FP, Djeunang R-M, Vemo BN, Moffo F and Djuissi Motchewo N (2020) Heat Induces Oxidative Stress: Reproductive Organ Weights and Serum Metabolite Profile, Testes Structure, and Function Impairment in Male Cavy (*Cavia porcellus*). *Front. Vet. Sci.*, 7:37. doi: 10.3389/fvets.2020.00037
- Odunitan-Wayas, F., Kolanisi, U. and Chimonyo, M. (2018). Haematological and Serum Biochemical Responses of Ovambo Chickens Fed Provitamin a Biofortified Maize. *Brazilian J. Poult. Sci.*, 20 (3): 425 - 434. <http://dx.doi.org/10.1590/1806-9061-2016-0444>
- Peters, S. O., Shoyebo, O. D., Ilori, B. M., Ebozoje, M.O., Ikeobi, C.O.N., and Adebambo, O.A. (2008). Semen quality traits of seven strain of chickens raised in the humid tropics. *Int. J. Poult. Sci.*, 7(10): 949-953.
- Rai, A., Snehashis Das, S., Chamallamudi, M. R., Nandakumar, K., Shetty, R., Gill, M., Sumalatha, S., Devkar, R., Gourishetti, K. and Kumar, N. (2018). Evaluation of the aphrodisiac potential of a chemically characterized aqueous extract of *Tamarindus indica* pulp. *J. Ethnopharmacol.*, 210: 118-124
- Razali, N., Sarni, M., Abdul Muthalib A. F., Subramaniam, S. and Abdul Aziz, A. (2012). Effects of various solvents on the extraction of antioxidant phenolics from the leaves, seeds, veins and skins of *Tamarindus indica* L. *Food Chem.*, 131: 441-448.
- Rezende, M. S., Mundim, A. V., Fonseca, B. B., Miranda, R. L., Oliveira Jr, W. and Lellis, C. G. (2017). Profile of Serum Metabolites and Proteins of Broiler

- Breeders in Rearing Age. *Brazilian J. Poult. Sci.*, 19 (4): 583-586. <http://dx.doi.org/10.1590/1806-9061-2016-0338>
- Saleh, B., Kalla, D. J. U., Mbap, S. T. and Doma, U. D. (2018). Effect of Varying Dietary Energy and Protein Levels on Semen Traits of FUNAAB – Alpha Roosters. *J. Anim. Prod. Res.*, 30(1):144-152.
- Saleh, B., Kalla, D. J. U., Mbap, S. T. and Doma, U. D. (2020). Effects of varying dietary energy and protein on testicular histomorphometry of caged FUNAAB – Alpha cocks. *Nig. J. Anim. Sci. Technol.*, 3(2):176-183
- Saleh, B., Kalla, D. J. U., Mbap, S. T., Doma, U. D. and Girgiri, A. Y. (2017). Effects of varying dietary energy and protein levels on gross morphology and histology of testes of breeder FUNAAB – Alpha cocks. *Nig. J. Anim. Prod.*, 44(2):25-33.
- Saleh, B., Mohammed, A. A., Yusuf, Z. A. and Musa, M. B. (2021). Effect of aqueous tamarind pulp extract on semen quality and testicular morphometry of Noiler Cocks in a hot dry environment. *Nig. J. Anim. Prod.*, 23 (1): 61-70.
- Swelum, A. A., Hashem, N. M., Abdelnour, S. A., Taha, A. E., Ohran, H., Khafaga, A.F., El-Tarabily, K. A. and Abd El-Hack, M. E. (2021). Effect of phytogetic feed additives on the reproductive performance animals. *Saudi J. Biol. Sci.*, 28: 5816 – 5822. <https://doi.org/10.1016/j.sjbs.2021.06.045>
- World Health Organization [WHO] (2010). WHO laboratory manual for the examination and processing of human semen. (5th ed.), WHO Press. Pp. 286.
- Zaniboni, L., Rizzi, R. and Cerolini, S. (2006). Combined effect of DHA and alpha tocopherol enrichment on sperm quality and fertility in the turkey. *Theriogenol.*, 65: 1813–1827.