EFFECT OF VARIED SUPPLEMENTAL ACETYLSALICYLIC ACID AND DIETARY SALT INCLUSION ON WEEKLY SEMEN QUALITY AND LEUKOGRAM PARAMETERS OF BROILER BREEDER COCKS RARE UNDER HEAT STRESS

OLOFIN, Adeyemi Avan, OLUWALADE, Oluwadunni Ruth, ALADE, Iyanuoluwa Tosin, ABDULKADIR, Muinat Titilope, ATOYEBI, Gbenga Oyewole and ARO, Samuel Olanrewaju
Department of Animal Production and Health, The Federal University of Technology, Ilesha-Akure Expressway, PMB 704, Akure, Ondo State, Nigeria.

Corresponding Authors: Olofin, A. A. Department of Animal Production and Health, The Federal University of Technology, Ilesha-Akure Expressway, PMB 704, Akure, Ondo State, Nigeria. **Email:** adeyemiolofin@gmail.com **Phone:** +234 816 417 9905

Received *July 05, 2024;* ***Revised*** *September 06, 2024;* ***Accepted*** *September 26, 2024*

ABSTRACT

This study investigated the optimum combination of acetylsalicylic acid (ASA) and salt (NaCl) in the Marshall Breeder cock that will synergistically improve weekly sperm cell and leukograms of this cocks. Sixteen broiler breeder cocks were used to determine the leukogram and weekly semen quality. Four diets were formulated and compared as: T_1 (0.25% NaCl and 0.00% ASA), T_2 (0.50% NaCl and 0.025% ASA), T_3 (0.75% NaCl and 0.050% ASA), and T_4 (1.00% NaCl and 0.075% ASA). The cocks were randomly distributed into four treatment diets with four replicates in each treatment and fed for sixteen weeks. Semen collection, through the abdominal massage method, commenced at the 30^{th} – 35^{th} week of age and was carried out once weekly between 6.00 and 8.00 hours. Diet T_3 showed a significant increase ($p<0.05$) in total ejaculate volume from 30 to 35 weeks of age with a peak at 33 weeks of age (0.6 ml), highest in sperm motility between the ranges of 98 – 100%, lowest in abnormal sperm cells (12 – 17%) and dead sperm cells (20 – 23%) when compared to other treatment diets. The leukogram showed that the heterophil and lymphocyte were significantly higher in diet T_3 (71.50 ± 1.36%) and lower in control diet T_1 (0.01 ± 0.01%), respectively. In conclusion, 0.750% NaCl worked synergistically with 0.050% ASA to improve the ejaculate characteristics of this breeder cocks from 30 to 35 weeks of age and could be used to optimise the concentration of heterophil and lymphocytes in the blood of breeder cocks.

Keywords: Acetylsalicylic acid, Salt, Marshall breeder cock, Semen, Sperm cell, Leukogram

INTRODUCTION

The most common environmental stressor working against the intensive livestock production sector in the tropics has been determined to be heat stress (Akinyemi *et al.,* 2019). It has been established to impair immune system function, which lowers broilers' performance concurrently (McDaniel and Parker, 2004). Several remedies have been used to mitigate the negative impact of heat stress on poultry performance, including the administration of potassium chloride (Ahmad *et al.,* 2008), sodium chloride (Dai *et al.*, 2009), ascorbic acid (Talebi and Khademi, 2011) and acetylsalicylic acid (Aro *et al.,* 2017).

Chicken sperm are produced in the intra-abdominal testes at a core body temperature (40 – 41 °C) for 14 days and then are transported through the epididymis to the vas deferens, where they are stored for 2 – 3 days and undergo

some natural degeneration before being exported at ejaculation (Pimprasert *et al.,* 2023). Considering this physiological evidence, it is reasonable to collect semen at a consistent frequency to maintain optimal sperm quality during fertility. More than three rounds of collections per week in turkeys decreased the semen volume (Noirault and Brillard, 1999). Acetylsalicylic acid (ASA) or aspirin is well-known as an antipyretic drug (Aro *et al.,* 2015). This drug has improved egg production and liveability in laying hens and prevents wet litter in poultry houses (Aro *et al.,* 2020). Since dietary sodium chloride (NaCl) has been reported to enhance egg production (Aro *et al.,* 2015) and boost the immune competence of farm animals (Aro *et al.,* 2015), it seems a worthwhile intervention strategy to experiment with these attributes of NaCl to improve semen production. There is, however, a paucity of data on the effects of aspirin and NaCl combination in the diet on the reproductive performance of broiler breeders, particularly males. The broiler breeder stock's infertility issues are inclined toward males (Silveira *et al.,* 2014). This infertility problem is exacerbated further, particularly in the tropics, by perennial heat stress (Abioja, 2010), linked to decreased spermatogenesis (Shadmehr *et al.,* 2018). The use of ASA and NaCl in combination with the poultry diet has been shown to lessen heat stress (Aro *et al.,* 2017) and reduce wet litter (Aro *et al.,* 2020). The optimal combination of ASA and NaCl in poultry diets, particularly in Marshall breeder broiler stock, is yet to be determined. Thus, the purpose of this study was to determine the best combination of ASA and NaCl in Marshall broiler breeder cocks to reduce heat stress while improving the semen production and leukogram indices of this breed of domestic chicken.

MATERIALS AND METHODS

Experimental Site: The experiment was conducted at the Poultry Unit of the Livestock Section of the Teaching and Research Farm, and Diagnostic Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure between November 2023 and March 2024.

Procurement and Management of Experimental Animals: Twenty broiler breeder cocks of the Marshall breed were purchased from Vettinson Breeders' Farms, Oyo State, Nigeria. The birds were twenty weeks of age at the time of their arrival. Sixteen birds with an average live weight of 2.2 kg were randomly allotted to four dietary treatments with four replicates in each treatment. Four treatment diets were formulated in which ASA was supplemented at 0, 250, 500 and 750 g/100kg and dietary salt inclusions at 250, 500, 750 and 1000 g/100kg of the diets. The diets were labelled as T1, T2, T3 and T4 respectively. Table 1 shows the gross composition of the Marshall breeder cock's diet.

Table 1: Gross composition (kg/100kg) of the experimental diets

Ingredients	T_I (kg)	T_2 (kg)	T_3 (kg)	T_4 (kg)
Maize	47.55	47.55	47.55	47.55
Wheat offal	16.00	16.00	16.00	16.00
Soybean meal	18.00	18.00	18.00	18.00
Rice bran	15.00	14.75	14.50	14.25
Lysine	0.20	0.20	0.20	0.20
Methionine	0.25	0.25	0.25	0.25
DCP	1.30	1.30	1.30	1.30
Limestone	1.20	1.20	1.20	1.20
Breeder's premix	0.25	0.25	0.25	0.25
Salt	0.25	0.50	0.75	1.00
Total	100.00	100.00	100.00	100.00
ASA	0.00	0.025	0.050	0.075
ME (Kcal/kg)	2700.93	2701.00	2701.07	2702.04
Crude protein (%)	16.01	16.08	16.15	16.22

T 1 = Diet with 0.25% NaCl and 0.00% ASA; T 2 = Diet with 0.50% NaCl and 0.025% ASA; T 3 = Diet with 0.75% NaCl and 0.050% ASA; T 4 = Diet with 1.00% NaCl and 0.075% ASA; ASA = Acetylsalicylic Acid; NaCl = Sodium Chloride; ME = Metabolizable Energy DCP = Dicalcium Phosphate and Breeder premix

Qualitative Semen Analysis: Total ejaculate volume was determined as described by Franken and Oehninger (2012). Sample bottles were used for semen collection per replicate from which semen or ejaculate volume for replicate was taken and recorded. Eosin-Nigrosin stains were used on smears for live/dead and abnormal/normal sperm cell counts. The smears were dried on a

warm slide and observed immediately with a light microscope at high power magnification (×100) (Franken and Oehninger (2012). Sperm motility was done by placing fresh semen on a cover slip and a drop of saline water was added which was then viewed under 40x magnification with a light microscope. Sperm concentration was calculated by multiplying the number of sperm cells counted by the dilution factor of the semen. The seminal volume and spermatocrit were determined using a centrifuging machine and calibrated pipette, the semen was aspirated, and the volume was read according to Franken and Oehninger (2012).

Differential Leukogram Count: A sample of the blood was smeared and then stained with Wright's dye and each type of white blood cell (lymphocytes, heterophil, monocytes, basophils, and eosinophil) was then counted under the microscope using a laboratory counter - Leucodiff 600 (Tagesu, 2018).

Statistical Analysis: All data obtained from the experiment were analysed using the Statistical Package for Social Sciences (SPSS) Version 26. Significant means were separated using the Duncan multiple-range test of the same statistical package.

RESULTS AND DISCUSSION

The control diet had the highest volume of ejaculate from Week 1 to 4 followed by a drastic reduction at Week 6 when compared with other diets in Figure 1.

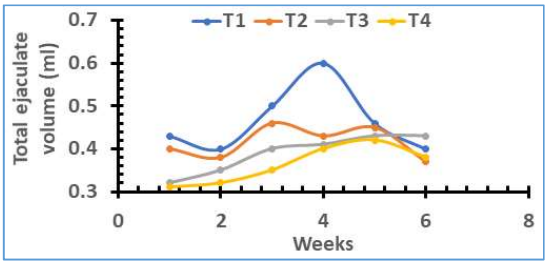

Figure 1: Weekly total ejaculate volume (%) produced by Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and varied inclusion of dietary salt. ***Key:*** *T_1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA*

Diet T_3 showed a progressive increase in the volume of the ejaculate from Week 1 to 6 with the highest ejaculate volume at Week 5 (0.55 ml) and 6 (0.53 ml) throughout the six-week study. The findings in this study align with the report made by Aro *et al.* (2020) that ASA when combined with NaCl at 0.05 and 0.50% of the diet would encourage maximum semen production of Isa White cocks. It shows that diet T_4 had the lowest spermatocrit value compared to other treatments. Throughout the study, the seminal plasma and spermatocrit (Figures 2 and 3) showed that diets supplemented with ASA-NaCl had their best at diet T_3 relative to the control diet.

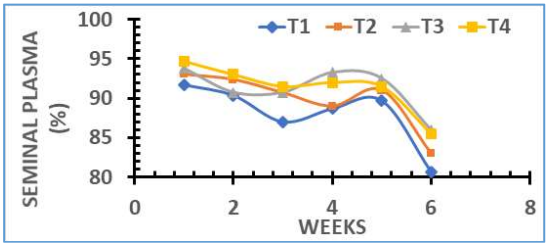

Figure 2: Weekly seminal plasma volume (%) produced by Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and varied inclusion of dietary salt. ***Key:*** *T_1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA*

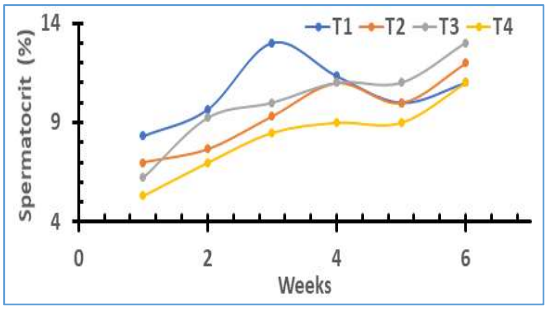

Figure 3: Weekly spermatocrit (%) of Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and varied inclusion of dietary salt. ***Key:*** *T_1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA*

The percentage of seminal plasma of birds fed diet T_3 (Figure 3) showed a gradual decrease from Week 1 to 6 compared to other treatment diets, while birds fed diet T_4 had the highest seminal plasma. Figure 3 shows an improving trend of spermatocrit from Week 1 to Week 6. This means that the less the seminal plasma the

lower the fluidity of the semen which eventually leads to an increase in spermatocrit percentage (Aro *et al.*, 2020). Furthermore, from weeks 3 to 6, diet T_3 showed a linear trend for sperm motility with a 100% rate while other treatment diets were lower (Figure 4).

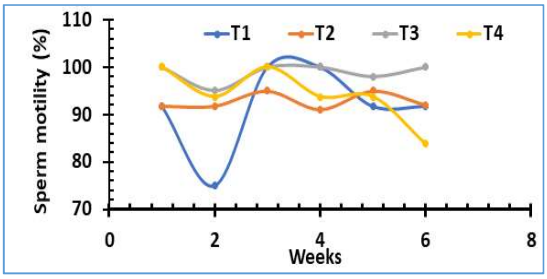

Figure 4: Weekly sperm motility (%) of Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and varied inclusion of dietary salt. ***Key:*** *T_1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA*

The fact that the birds were rare during the upsurge of heat period (November to March) and as well as exposed to frequent semen collection, at this level of supplementation of both dietary ASA and NaCl, these indices were improved when compared to the control diet.

In Figure 5, it was observed that T_3 had the lowest percentage of dead sperm cells. At Week 6, however, all treatments had a higher percentage of dead sperm cells compared to Week 1 of the semen collection period except diet T_3. Diets T_1 and T_4 were observed to have the same percentage of dead sperm cells at Week 1(25%), 4(29.25%) and Week 6(30%).

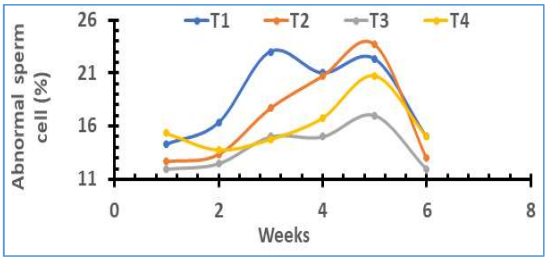

Figure 5: Weekly abnormal sperm cells (%) in Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and varied inclusion of dietary salt. *Key: T1 = Diet with 0.25% NaCl and 0.00% ASA; T2 = Diet with 0.50% NaCl and 0.025% ASA; T3= Diet with 0.75% NaCl and 0.050% ASA; T4 = Diet with 1.00% NaCl and 0.075% ASA*

Cocks fed diet T_2 recorded 24% dead sperm cells in Week 1 which increased at Week 3 to 32.67%, fell to 20% in Week 5 and rose to 27% in Week 6.

According to Figure 6, the general trend observed was a progressive increase in abnormal sperm cells of the cocks' fed diets with NaCl-inclusions and ASA supplementation from Week 1 to Week 5 followed by a reduction at Week 6.

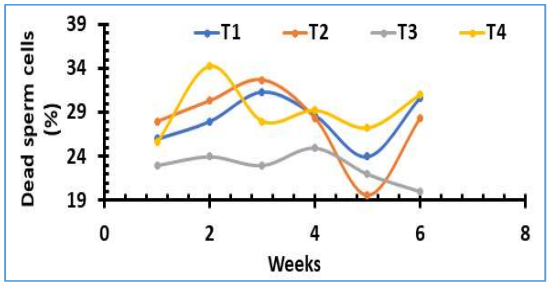

Figure 6: Weekly dead sperm cells (%) in Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and varied inclusion of dietary salt. *Key: T_1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA*

Cocks fed diet T_3 had the lowest percentage of abnormal sperm cells throughout the study period with its lowest record at Week 1(12%) and 6(12%). The control diet T_1 had the highest percentage of dead sperm cells at Week 2(16.33%), 3(23%) and 4(21%) while diet T_2 was the highest at Week 5(23.67%).

The best improvement of sperm motility, reduced in dead sperm cells and abnormal sperm cells observed in diet T_3 may be due to Na^+ in the salt providing the energy needed for sperm motility, as they play a role in the process of ATP synthesis, which is the primary source of energy for sperm movement (Guyton and Hall, 2006). Furthermore, Na^+ is involved in the regulation of fluid balance within sperm cells, which is essential for maintaining structural integrity and protecting it from damage (Guyton and Hall, 2006). In addition, aspirin, known for its anti-stress and anti-inflammatory activity, could have reduced any oxidative stress within the seminal plasma, avoided inflammation, and thus contributed to the improvement of those ejaculate parameters (Fan *et al.,* 2013). It was observed that the sperm cell concentration increased from Week 1 to Week 5 among all the treatment diets and decreased at Week 6 (Figure 7).

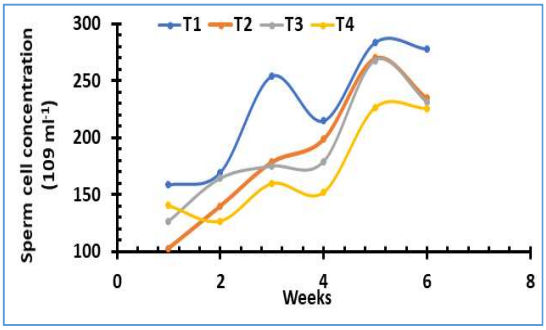

Figure 7: Weekly sperm cell concentration (%) of Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and varied inclusion of dietary salt. *Key:* *T_1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA*

The reduction in the sperm concentration values of the birds fed ASA-supplemented and dietary NaCl-inclusion diets agrees with Vyas *et al.* (2016) who noted a decrease in sperm concentration of rats due to aspirin, causing androgen depletion in the target level, particularly in the caudal epididymis of rat, thereby affecting physiological maturation of the sperm.

Leukogram Parameters of Marshall Broiler Breeder Cocks Fed Dietary Supplementation of Acetylsalicylic Acid (ASA) and Dietary Salt Levels: Results of leukogram of Marshall Broiler breeder cocks fed supplemental acetylsalicylic acid and dietary salt inclusions are as given in Table 2. According to this experimental study, it was observed that the eosinophil, monocyte and heterophil were recorded as not significantly different ($p>0.05$) among all the treatment diets. However, there were significant differences ($p<0.05$) in the heterophil among the experimental birds with diet T_3 having the lowest (0.01 ± 0.01%) while the highest in the percentage of lymphocytes (71.50 ± 2.36%). Moreover, the percentage of basophil was measured to be significantly highest in the birds placed on diet T4 (0.45 ± 0.11%) while the lowest was found in diet T_3 (0.26 ± 0.06%).

Animals with low white blood cells are exposed to a high risk of disease infection, while those with moderate counts can generate antibodies in the process of phagocytosis and have a high degree of resistance to diseases (Soetan *et al.,* 2013) and enhance adaptability to local environmental and disease prevalent conditions (Isaac *et al.,* 2013).

Table 2: Leukogram parameters of Marshall Broiler breeder cocks fed dietary supplementation of acetylsalicylic acid (ASA) and dietary salt levels

Parameters	T1	T2	T3	T4
BAS (%)	0.39 ± 0.06^{b}	0.30 ± 0.10^{a}	0.26 ± 0.06^{a}	0.45 ± 0.11^{b}
EOS (%)	0.85 ± 0.11	0.79 ± 0.15	0.70 ± 0.11	0.80 ± 0.15
LYM (%)	60.67 ± 4.62	63.67 ± 3.36	71.50 ± 1.36	64.29 ± 3.97
HET (%)	0.06 ± 0.09^{b}	0.01 ± 0.02^{a}	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}
HET/LYM (%)	0.53 ± 0.09^{b}	0.41 ± 0.02^{ab}	0.34 ± 0.01^{a}	0.49 ± 0.01^{ab}
MON (%)	2.05 ± 1.15	1.33 ± 0.67	1.25 ± 0.85	2.00 ± 0.58

a, b = Means on the same rows but with different letter superscripts are statistically significantly different ($p<0.05$); T_1 = Diet with 0.25% NaCl and 0.00% ASA; T_2 = Diet with 0.50% NaCl and 0.025% ASA; T_3= Diet with 0.75% NaCl and 0.050% ASA; T_4 = Diet with 1.00% NaCl and 0.075% ASA. BAS = Basophils; EOS = Eosinophils; LYM = Lymphocytes; HET = Heterophils and MON = Monocytes

In general, an overwhelmingly high WBC count in the peripheral blood is often observed in stress and inflammatory conditions due to generalized or localized infections, trauma, toxicities, neoplasms, and so on (Doneley and Doneley, 2010).

In this study, the highest white blood cell parameters indicative of stress were found in the control diet. These birds had the highest percentage of heterophils and the lowest level of lymphocytes. Lymphocyte levels are reduced in response to elevated stress (Peters *et al.,* 2011). In turn, the number of heterophils increases in response not only to pathogens (bacteria, fungi) but also to stress (Dhabhar, 2009), with a simultaneous decrease in the number of lymphocytes (Feldman *et al.,* 2000). The heterophil-to-lymphocyte ratio is considered a reliable indicator of the stress level in birds, primarily making it possible to determine the body's response to stress caused by the environment (Davis *et al.,* 2008)

The values of this parameter indicate that the control diet T_1 had the highest level of

stress. It can be deduced that ASA could work in synergy with NaCl at these combinational levels to combat any form of stress in this animal.

Conclusion: All the ejaculate parameters differed in trend across treatments throughout the six-week study period. Diet T_3, had the best total ejaculate volume, and sperm motility and reduced the dead/abnormal sperm cells and could be used to optimize the concentration of heterophils and lymphocytes in the blood of heat-stressed chickens. As a result, the usage of ASA supplementation and dietary NaCl inclusion at combinations of 0.75% NaCl: 0.05% ASA in this current experimental study improves the reproductive parameters and leukogram indices of breeder cocks.

ACKNOWLEDGEMENTS

The authors thank the technical staff of the Poultry Unit of the Livestock Section of the Teaching and Research Farm, and Diagnostic Laboratory of the Department of Animal Production and Health, Federal University of Technology, Akure for their various contributions. The authors also appreciate the effort of the anonymous reviewers for their various critics that positively shaped this publication.

REFERENCES

ABIOJA, M. O. (2010). *Temperature-Humidity Effects on Egg Fertility and Evaluation of Vitamin C and Cold Water on Broiler Growth in Hot Season*. A PhD Thesis Submitted to the Department of Animal Physiology, University of Agriculture, Abeokuta, Nigeria.

AHMAD, T., KHALID, T., MUSHTAQ, T., MIRZA, M. A., NADEEM, A., BABAR, M. E. and AHMAD, G. (2008). Effect of potassium chloride supplementation in drinking water on broiler performance under heat stress conditions. *Poultry Science*, 87(7): 1276 – 1280.

AKINYEMI, M. O., OSAMEDE, O. H. and EBOREIME, A. E. (2019). Effects of heat stress on physiological parameters and serum concentration of HSP70 in indigenous breeds of sheep in Nigeria. *Slovak Journal of Animal Science*, 52(03): 119 – 126.

ARO, S. O., FALUYI, O. B., AWONEYE, O. O. and ONIBI, G. E. (2020). Dietary supplementation of acetylsalicylic acid improved growth, livability and egg production performance in two breeds of commercial layers. *Nigerian Journal of Animal Production*, 47(2): 151 – 160.

ARO, S. O., OSHO, I. B. and AWONEYE, O. O. (2017). Comparison of rectal and axillary temperatures of Isa Brown and Harco Black layers fed different levels of dietary acetylsalicylic acid. *Animal Research International*, 14(1): 2691 – 2696.

ARO, S. O., OWOKOTOMO, E. P. and ADEBAYO, A. D. (2015). The effects of varying levels of dietary salt on performance and faecal parameters of late production layers. Pages 407 – 410. *In: Proceedings of the 40th Annual Conference of the Nigerian Society for Animal Production*, 15 - 19th March 2015, National Animal Production Research Institute (NAPRI)/Amadu Bello University, Zaria.

DAI, N. V., BESSEI, W. and QUANG, N. H. (2009). The effects of sodium chloride and potassium chloride supplementation in drinking water on performance of broilers under tropical summer conditions. *European Poultry Science (EPS),* 73(1): 41 – 48.

DAVIS, A. K., MANEY, D. L. and MAERZ, J. C. (2008). The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Functional Ecology*, 22(5): 760 – 772.

DHABHAR, F. S. (2009). Enhancing versus suppressive effects of stress on immune function: implications for immunoprotection and immunopathology. *Neuroimmunomodulation*, 16(5): 300 – 317.

DONELEY, B. and DONELEY, R. (2010). *Avian Medicine and Surgery in Practice: Companion and Aviary Birds.* Manson Publishing Limited, London, United Kingdom.

FAN, Y. P., TANG, J. J., LU, H., ZHANG, Y. C., RUAN, J. L., TENG, X. M. and HAN, Y. B.

(2013). Progesterone induction keeps a balanced mitochondrial activity and a low ROS productivity in human sperm. *National Journal of Andrology*, 19(10): 880 – 885.

FELDMAN, B., ZINKI, J. and JAIN, N. (2000). *Schalm's Veterinary Haematology*. 5th Edition, Lippincott and Wilkins, Philadelphia, USA.

FRANKEN, D. R. and OEHNINGER, S. (2012). Semen analysis and sperm function testing. *Asian Journal of Andrology*, 14(1): 6 - 13.

GUYTON, A. C. and HALL, J. E. (2006). *Textbook of Medical Physiology*. Elsevier Saunders, Philadelphia, Pennsylvania, USA.

ISAAC, L. J., ABAH, G., AKPAN, B. and EKAETTE, I. U. (2013). Haematological properties of different breeds and sexes of rabbits. *Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria,* 2013: 24 – 27.

MCDANIEL, C. and PARKER H. M. (2004). The effects of dietary acetylsalicylic acid on heat stress infertility of broiler breeder males. *International Journal of Poultry Science*, 3(9): 570 – 577.

NOIRAULT, J. and BRILLARD, J. P. (1999). Effects of frequency of semen collection on quantitative and qualitative characteristics of semen in turkey breeder males. *Poultry Science*, 78(7): 1034 – 1039.

PETERS, S. O., GUNN, H. H., IMUMORIN, I. G., AGAVIEZOR, B. O. and IKEOBI, C. O. N. (2011). Haematological studies on frizzled and naked neck genotypes of Nigerian native chickens. *Tropical Animal Health and Production*, 43: 631 – 638.

PIMPRASERT, M., KHEAWKANHA, T., BOONKUM, W. and CHANKITISAKUL, V. (2023). Influence of semen collection frequency and seasonal variations on fresh and frozen semen quality in Thai native roosters. *Animals*, 13(4): 573. https://doi.org/10.3390/ani13040573

SHADMEHR, S., TABATABAEI, S. R. F., HOSSEINIFAR, S., TABANDEH, M. R. and AMIRI, A. (2018). Attenuation of heat stress-induced spermatogenesis complications by betaine in mice. *Theriogenology*, 106: 117 – 126.

SILVEIRA, M. M., DE FREITAS, A. G., MORAES, C. A., GOMES, F. S., LITZ, F. H., MARTINS, J. M. S., FAGUNDES, N. S. and FERNANDES, E. A. (2014). Feeding management strategy for male broiler breeders and its effects on body weight, hatchability and fertility. *Brazilian Journal of Poultry Science*, 16(4): 397 – 402.

SOETAN, K. O., AKINRINDE, A. S. and AJIBADE, T. O. (2013). Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (*Sorghum bicolor*). *Proceedings of 38th Annual Conference of Nigerian Society for Animal Production,* 2013: 49 – 52.

TAGESU, A. (2018). *Manual Guidance of Veterinary Clinical Practice and Laboratory*. International Journal of Veterinary Science and Research Special Issue, Peertechz Publications, Private Limited, India.

TALEBI, E. and KHADEMI, M. (2011). Combination effects of ascorbic acid and glucose in drinking water on the broiler performance under acute heat stress. *International Journal of Applied Biology and Pharmaceutical Technology,* 2(1): 92 – 96.

VYAS, A., RAM, H., PUROHIT, A. and JATWA, R. (2016). Adverse effects of subchronic dose of aspirin on reproductive profile of male rats. *Journal of Pharmaceutics*, 2016(1): 6585430. https://doi.org/10.1155/2016/6585430