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Haematology, blood viscosity and osmotic fragility of finisher broiler chickens fed dietary supplementation of acetylsalicylic acid during hot dry season

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ABSTRACT: The effect of acetylsalicylic acid on the haematological status, blood viscosity and osmotic fragility of broiler chickens were evaluated during transition from dry to rainy season (January – March). One hundred and ninety two (192) day old broiler chicks (ANAK) were randomly divided into four dietary treatments, each treatment comprising 48 birds, which was replicated 4 times containing 12 birds per replicate. Four experimental diets were formulated to contain acetylsalicylic acid (ASA) at 0, 0.025, 0.050 and 0.075 percentage; the diets were formulated to meet the nutrient requirement of the birds. All data collected were subjected to one-way analyses of variance (ANOVA) using a completely randomized design. The result of the hematological study showed that the packed cell volume, red blood cell, erythrocyte sedimentation rate, mean cell volume, mean cell haemoglobin and heretrophil/lymphocyte ratio were influenced by the treatment diets. Broilers fed 0.075% ASA had the highest packed cell volume and red blood cell values of 29.63±0.77% and 2.60±0.17×10^6 mm respectively. The blood viscosity study revealed that the whole blood viscosity decreased as level of ASA supplementation increased. Broilers fed 0.050% ASA had the lowest ($p<0.05$) serum and plasma viscosity values of 1.05±0.03 cP and 0.72±0.01 cP, respectively. Statistical variations ($p<0.05$) were observed at all levels of saline concentration across the treatment, with broilers fed 0.075% having the highest number of osmotically stable red blood cells across the treatments at all saline concentrations. It could be concluded that birds fed ASA supplemental diets were less viscous, osmotically stable and had a better haematological profile than the control diet.

Keywords: Acetylsalicylic acid, broiler, fragility, haematology, viscosity.

INTRODUCTION

The immune response of animal is modulated by central nervous system through a complex network between the nervous, endocrine and immune systems, it is altered through the hypothalamic-pituitary-adrenal (HPA) and the sympathetic-adrenal medullar (SAM) axes (Marketon and Glaser, 2008). In poultry, studies have investigated the effect of heat stress on the haematological profile of poultry species, and all studies showed an immunosuppressing effect of heat stress on broilers and laying hens. Aengwanich (2008) demonstrated decreased number of lymphocytes in the cortex and medulla areas of the bursa. Research findings have also revealed alteration of levels of circulating cells by increasing heterophil : lymphocyte ratio, due to reduced numbers of circulating lymphocytes and higher numbers of heterophils (Bartlett and Smith, 2003; Niu *et al.*, 2009; Quinteiro-Filho *et al.,* 2010; Quinteiro-Filho *et al.,* 2012). Oladele *et al.* (2003) reported significantly low levels of packed cell volume of 23.73 ± 0.12% in domestic chickens during the hot-dry season, while the values of 24.82 ± 0.49% and 24.63 ± 0.10% were obtained during the harmattan and rainy seasons, respectively. The low values of haemoglobin and packed

cell volume recorded during the hot-dry season in the zone were attributed to heat and nutritional stress, which impair the synthesis of blood cells in birds (Oladele *et al*., 2001). Aspirin or acetyl salicylic acid (ASA) is a derivative of salicylate, it is generally used as an analgesic, antipyretic, and anti-inflammatory drug. Its ability to control fever is due to its action on the prostaglandin system, by irreversible inhibiting the enzyme cyclo-oxygenase COX (Bartfai and Conti, 2010) which is required for prostaglandin and thromboxane synthesis, this thus produces an inhibitory effect on platelet aggregation during the lifetime of the affected platelet. This antithrombotic property makes aspirin useful for reducing the incidence of heart attacks (Seshasai *et al*., 2012; McNeil *et al.,* 2018). It has been demonstrated that feeding ASA to chickens during heat stress lowers body temperature. Aro *et al*. (2017a) revealed that administration of ASA to layer birds reduced the evening axillary temperature by 0.26°C and evening rectal temperature by 0.18°C. Also, Al-Obaidi and Al-Shadeedi (2010) reported that feeding of 0.2% ASA to broilers increased live body weight. Thus, these attributes of aspirin as anti-platelet, anti-pyretic, analgesia and anti-inflammatory drug have necessitated this research work to investigate the blood profile of broiler chickens fed dietary supplementation of acetylsalicylic acid during hot dry season.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Teaching and Research Farm (poultry unit) of the Rufus Giwa Polytechnic Owo (RUGIPO (Latitude 7°11'46.32"N and longitude 5°35'12.52"E), Ondo State, Nigeria. The experimental site is located in southwestern Nigeria, where the climate is influenced by the rain-bearing Southwest monsoon winds from the Atlantic Ocean and dry Northeast trade wind from the Sahara Desert. The rainy season lasts for about seven months with about 1524 mm of rainfall per annum. The atmospheric temperature ranges between 26°C and 31°C, with mean annual relative humidity of about 80% (Climatic Data, 2018).

Procurement of dietary supplement

Aspirin (Acetylsalicyclic acid) was purchased from a reputable Pharmaceutical store in Owo, Ondo State, Nigeria.

Experimental animals and feeding

One hundred and ninety two (192) day old broiler chicks (ANAK) were purchased from a reliable source. The birds were raised in a deep litter system using 2.5 m x 2.5 m pen size. Feed and water were supplied *ad libitum* and good hygienic condition was maintained throughout the period of feeding trial of 8 weeks. Four treatment diets were formulated. Diet 1 (T1) which is the control had no inclusion of acetylsalicylic acid (ASA), Diet 2 (T2) contained 0.025% inclusion of ASA, Diet 3 (T3) contained 0.050% inclusion of ASA and Diet 4 (T4) had 0.075% of ASA. Tables 1 and 2 show the gross composition of the broiler starter and broiler finisher' diets respectively.

Experimental layout and feeding trial

The birds were randomly divided into four dietary treatments, each treatment comprising 48 birds, which was replicated 4 times containing 12 birds per replicate.

Slaughtering of the experimental birds

At the end of the 8 weeks feeding trial, the final weight was taken; three birds per replicate were randomly selected, stunned and slaughtered.

Data collection

Blood samples (5 mls of blood from each chicken) were collected from the birds into sterile glass tubes containing EDTA (Ethylene Diamine Tetra Acetic Acid). Blood samples were agitated slightly to avoid coagulation in the sample bottle and the following haematological parameters were investigated; the erythrocyte count (RBC) $x10^6$ mm (Campbell and Ellis, 2007), packed cell volume (PCV) % Rehman *et al*. (2003), haemoglobin (Hb) concentration (g/100ml) (Drabkin, 1945), mean corpuscular values (MCV (u^3), MCH (pg), MCHC%) and total leukocyte count. Percent heterophils %, lymphocytes %, eosinophils %, monocytes % and basophils % were also determined according to the technique described by Campbell and Ellis (2007). The viscosities were determined with the use of viscometer and stop watch. The values of the whole blood, serum and plasma viscosity were calculated from the values obtained from the viscometer reading using the formular:

$$\text{Viscosity} = \frac{\text{Flow time of sample x 1.0038}}{\text{Flow time of water}}$$

Where 1.0038 is the viscosity of water at standard temperature and pressure and the flow time of water is 2.74 seconds (Aro and Akinleminu, 2015).

Erythrocyte osmotic fragility (EOF) test was carried out as described by Oyewale (1991) using NaCl and distilled

Table 1. Composition (g/100g) of broiler starter diets (0-4 Weeks) during the transition from dry to rainy season (January - March).

Ingredients	Acetylsalicylic acid (%)			
	T_1 (0.00%)	T_2 (0.025%)	T_3 (0.050%)	T_4 (0.075)
Maize	52.00	52.00	52.00	52.00
Wheat Offal	5.00	5.00	5.00	5.00
Groundnut Cake	16.00	16.00	16.00	16.00
Soya Bean Meal	21.40	21.40	21.40	21.40
Fish Meal	1.50	1.50	1.50	1.50
Bone meal	1.50	1.50	1.50	1.50
Limestone	1.00	1.00	1.00	1.00
Lysine	0.10	0.10	0.10	0.10
Methionine	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Acetylsalicylic acid (ASA)	0.00	0.025	0.050	0.075
Calculated analysis				
Crude Protein (%)	23.10	23.10	23.10	23.10
Metabolizable Energy (MJ/Kg)	11.78	11.78	11.78	11.78
Ether Extract (%)	4.67	4.67	4.67	4.67
Crude Fibre (%)	3.92	3.92	3.92	3.92
Phosphorus (%)	0.72	0.72	0.72	0.72
Calcium (%)	1.03	1.03	1.03	1.03
Lysine (%)	1.19	1.19	1.19	1.19
Methionine (%)	0.85	0.85	0.85	0.85

T1 = Diet with 0.00%ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050%ASA; T4 = Diet with 0.075% ASA.

water. 100 ml of distilled water was measured into each of 10 test tubes. 0.0 to 0.9 g of NaCl was measured and dissolved into each of the test tube to give a saline concentration that ranged from 0.0 to 0.9 g per 100 ml of distilled water. One milliliter (1 ml) of blood collected from the birds was added to saline solution in the test tubes from which the percentage of red blood cells haemolysed per saline concentration was calculated and used as a measure of red cells osmotic fragility.

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using a completely randomized design (CRD) of SAS (2008) statistical package. Duncan's multiple range test of the same statistical package was used to compare the means.

RESULTS

Haematological indices of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (February- April) is as shown in Table 3. The erythrocyte sedimentation rate, packed cell volume, red blood cell, heterophil/lymphocyte ratio, mean cell volume and mean cell haemoglobin were influenced by the treatment diets. Broilers fed 0.075% ASA had the highest packed cell volume and red blood cell values of 29.63±0.77% and 2.60±0.17×10^6 mm, respectively. The mean cell volume and mean cell haemoglobin were observed to be high in broilers fed control diet. The result also revealed that erythrocyte sedimentation rate, mean cell volume and mean cell haemoglobin values decreased as the level of ASA supplementation increased.

The blood viscosity of finisher broiler chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (February- April) is as shown in Table 4. The whole blood, plasma and serum viscosities were significantly influenced ($p<0.05$) by the dietary treatments. The blood viscosity study revealed that the whole blood viscosity decreased as level of ASA supplementation increased. Broilers fed 0.050% ASA had the lowest ($p<0.05$) serum and plasma viscosity values of 1.05±0.03 cP and 0.72±0.01 cP respectively.

Table 2. Composition (g/100g) for broiler finisher's diets (5th-8th week) during the transition from dry to rainy season (January -March)

Ingredients	Acetyl salicylic acid (%)			
	T_1 (0.00%)	T_2 (.025%)	T_3 (0.050%)	T_4 (0.075%)
Maize	62.00	62.00	62.00	62.00
Wheat Offal	4.70	4.70	4.70	4.70
Groundnut Cake	17.20	17.20	17.20	17.20
Soya Bean Meal	11.00	11.00	11.00	11.00
Fish Meal	1.00	1.00	1.00	1.00
Bone meal	1.50	1.50	1.50	1.50
Limestone	1.00	1.00	1.00	1.00
Lysine	0.20	0.20	0.20	0.20
Methionine	0.40	0.40	0.40	0.40
Salt	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Acetylsalicylic acid (ASA)	0.00	0.025	0.050	0.075
Calculated Analysis				
Crude Protein (%)	19.60	19.60	19.60	19.60
Metabolizable Energy (MJ/Kg)	12.21	12.21	12.21	12.21
Ether Extract (%)	4.76	4.76	4.76	4.76
Crude Fibre (%)	3.53	3.53	3.53	3.53
Phosphorus (%)	0.68	0.68	0.68	0.68
Calcium (%)	1.00	1.00	1.00	1.00
Lysine (%)	1.03	1.03	1.03	1.03
Methionine (%)	0.70	0.70	0.70	0.70

T1 = Diet with 0.00%ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050%ASA; T4 = Diet with 0.075% ASA.

Table 3. Haematological indices of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (February- April).

Parameters	T1	T2	T3	T4
ESR (mm/hr)	4.25±0.66 ^{a}	4.13±0.55 ^{a}	3.75±0.25 ^{a}	2.63±0.47^{b}
PVC (%)	27.50±0.89 ^{c}	28.00±0.79^{bc}	28.38±0.38 ^{b}	29.63±0.77 ^{a}
RBC (x10^{6}mm	2.28±0.30 ^{b}	2.20±0.21^{c}	2.30±0.07 ^{b}	2.60±0.17 ^{a}
HB (g/100ml)	9.15±0.30	9.31±0.26	9.45±0.13	9.88±0.26
Lymphocyte (%)	59.75±0.25	60.25±0.43	59.50±0.61	59.75±0.25
Heterophyl (%)	25.13±0.43	24.00±0.91	24.63±0.38	24.50±0.54
Heterophil: Lymphocyte	2.38±0.01^{b}	2.52±0.01^{a}	2.42±0.01^{ab}	2.44±0.00^{ab}
Monocyte (%)	12.13±0.43	12.13±0.66	12.63±0.55	12.25±0.48
Eosinophyils (%)	2.25±0.14	2.38±0.34	2.13±0.13	2.50±0.20
Basophyils (%)	0.88±0.13	1.25±0.14	1.25±0.14	1.00±0.00
MCV (u^{3})	131.46±6.46 ^{a}	129.61±7.96^{ab}	124.83±3.07^{ab}	115.47±4.98 ^{c}
MCH (pg)	43.73±2.12 ^{a}	43.10±2.66 ^{a}	41.56±0.97 ^{b}	38.48±1.63 ^{c}
MCHC (%)	33.27±0.03	33.25±0.01	33.30±0.05	33.34±0.03

a, ab, b, bc, c= Means on the same rows but with different superscripts are statistically ($P<0.05$) significant. T1 = Diet with 0.00% ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050% ASA; T4 = Diet with 0.075% ASA; ESR = Erythrocyte Sedimentation Rate; PCV=Packed Cell Volume; RBC= Red Blood Cell; HB=Haemoglobin; MCV=Mean Cell Volume; MCHC= Mean cell haemoglobin concentration; MCH=Mean cell haemoglobin.

Table 4. Blood viscosity of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (February- April).

Parameters	T1	T2	T3	T4
Whole Blood (cP)	2.51±0.07 [a]	2.24±0.09[b]	2.18±0.04[b]	2.09±0.07[b]
Serum (cP)	1.11±0.05 [a]	1.05±0.04 [b]	1.05±0.03 [b]	1.10±0.03 [a]
Plasma (Cp)	1.02±0.13 [a]	1.00±0.07 [a]	0.72±0.01[b]	1.01±0.07 [a]

a, b = Means on the same rows but with different superscripts are statistically (P<0.05) significant. T1 = Diet with 0.00% ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050% ASA; T4 = Diet with 0.075% ASA; cP = centiPoise.

Table 5. Osmotic stability of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (February- April).

Saline concentration (%)	T1	T2	T3	T4
0.0	1.30±0.17[c]	1.44±0.17[b]	1.48±0.06[b]	1.64±0.11[a]
0.1	1.38±0.17[d]	1.51±0.17[c]	1.61±0.15[b]	1.74±0.08[a]
0.2	1.48±0.16[c]	1.59±0.17[b]	1.63±0.06[b]	1.82±0.10[a]
0.3	1.56±0.18[c]	1.66±0.17[bc]	1.71±0.06[b]	1.93±0.10[a]
0.4	1.63±0.18[c]	1.73±0.17[b]	1.80±0.07[b]	2.04±0.11[a]
0.5	1.73±0.19[c]	1.80±0.17[bc]	1.88±0.07[b]	2.12±0.11[a]
0.6	1.83±0.19[bc]	1.91.±0.17[b]	1.99±0.06[a]	2.23±0.12[a]
0.7	1.92±0.19[c]	2.01±0.18[b]	2.09±0.07[b]	2.33±0.13[a]
0.8	2.06±0.20[c]	2.14±0.18[bc]	2.21±0.07[b]	2.45±0.12[a]
0.9	2.13±0.20[c]	2.19±0.18[c]	2.28±0.07[b]	2.48±0.13[a]

[a, b, bc, c] = Means on the same rows but with different superscripts are statistically (P<0.05) significant. T1 = Diet with 0.00% ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.050% ASA; T4 = Diet with 0.075%.

Table 5 shows the osmotic stability of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid at the transition from dry to rainy season (February-April). Statistical variations (p<0.05) were observed at all levels of saline concentration across the treatment, with broilers fed 0.075% having the highest number of osmotically stable red blood cells across the treatments at all saline concentrations. It was observed that the number of red blood cell that were osmotically stable increased as the level of ASA supplementation increased. Similarly, the result also revealed that the number of osmotically stable red blood cell increased as saline concentration increased.

DISCUSSION

The result of the haematological study of broiler finisher chickens fed dietary supplementation of acetylsalicylic acid during transition period from dry to rainy shows that broilers fed 0.075% ASA had the highest packed cell volume and red blood cell values, the high packed cell volume and red blood cell values recorded in broiler fed 0.075% ASA could be attributed to the ability of ASA to improve the feed intake, feed efficiency, and absorption of nutrients has been reported by many researchers (Laura *et al*., 2009; Al-Obaidi and Al-Shadeedi, 2010; Amao and Siyanbola, 2012; Aro *et al*., 2017b), this invariably enhances the process of haematopoies is from the bone marrow. The whole blood, plasma and serum viscosities were significantly influenced (p<0.05) by the dietary treatments.

The whole blood viscosity decreased as level of ASA supplementation increased. Broilers fed 0.050% ASA had the lowest (p<0.05) serum and plasma viscosity value. The low viscosity recorded in broilers fed 0.050% ASA is a pointer that the blood of broilers in this group was thinner and this could be attributed anti-platelet attribute of ASA by disrupting prostaglandin synthesis in thrombocytes (Balog *et al.,* 2000; Bode-Böger,2005; Tauseef *et al.,* 2008).

Significant differences (p<0.05) were observed at all levels of saline concentration across the treatment. It was observed that the number of red blood cell that were osmotically stable increased as the level of ASA supplementation increased with broilers fed 0.075% having the highest number of osmotically stable red blood cells. The high number of red blood cell that were osmotically stable in ASA supplemented diets showed that ASA has the capacity of increasing the resistance of erythrocyte membrane to haemolysis (Aldrich *et al.*, 2006;

Adenkola *et al.,* 2010). The result obtained in this study is in contrast to the report of Oladele *et al.* (2003), Adenkola and Ayo (2009b) and Adenkola *et al.* (2010) who reported significant decrease in erythrocyte osmotic stability during the harmattan season, when pigs were exposed to road transportation stress.

Conclusion

It could be concluded that inclusion of acetylsalicylic acid improves the haematological status of the experimental broilers. Birds fed ASA supplemental diet had low ($p<0.05$) blood viscosity and were osmotically stable than the control diet, hence, supplementation of ASA into the diets of domestic chickens for a period of 8 weeks enhances blood profile of broiler chickens.

CONFLICT OF INTEREST

The authors declare that they have no conflict interest.

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