OSMOTIC FRAGILITY, CIRCULATING LUTEINIZING AND STEROIDAL HORMONES IN LAYING CHICKENS FED DIETARY INCLUSION OF ACETYLSALICYLIC ACID (ASA)

S. O. ARO, O. I. BAKI AND O. O. AWONEYE
Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria.
Correspondence:soaro@futa.edu.ng;07062329254

ABSTRACT
This experiment investigated the effect of feeding ASA on osmotic fragility, circulating luteinizing and steroidal hormones in laying hens. Four diets varying in ASA levels of 0.00%, 0.025%, 0.050% and 0.075% were formulated and fed to 192 laying chickens in a 16-week trial. The birds were allotted randomly into the four treatment diets at 48 birds per treatment with each treatment replicated four times at 12 birds per replicate. Results from blood samples collected at the end of the experiment showed significant differences in the erythrocytic fragility between the Isa Brown (IB) and Harco Black (HB) breeds. IB was more osmotically stable to erythrocytic fragility than the HB breed at all saline concentrations. There was however no treatment or interaction effect on the erythrocytic fragility of the bird. While the four treatment diets did not show a clear–cut trend on their effects on the osmotic fragility of the red blood corpuscles (rbc), the interaction between the breed and the treatment showed an increase in osmotic stability of rbc of the HB with increase in ASA level while IB breed showed a decrease in osmotic stability of rcb with increase in the levels of ASA in the diets. It therefore meant that the IB breed would be more osmotically liable to lysis at higher ASA concentration than the HB breed. The effect of ASA on circulating pituitary and steroidal hormones revealed that there were breed differences in estradiol (E2) and testosterone concentration with the HB recording a higher ($P<0.05$) E2 (440.38±92.62pmol/L) versus (229.37±84.10pmol/L) and testosterone (0.68±0.11nmol/L versus 0.12±0.08nmol/L) than the IB breed. There was significant treatment effect of ASA on progesterone, testosterone and E2 concentration in which these three parameters showed gradual increase with increase in dietary levels of ASA. The effects of breed versus treatment revealed that all the four parameters viz; progesterone, testosterone, E2 and cortisol differed significantly. Both IB and HB showed a corresponding increase with increase in the levels of ASA in the feed. The HB breed however gave a more consistent increase in the circulating serum values for all these parameters than in the IB breed. Conclusively, IB breed was the more osmotically stable breed in terms of the fragility of red blood corpuscles but could be more sensitive to fragility than HB breed at higher ASA concentrations. The use of ASA in the diets of laying chickens could potentiate progesterone, testosterone and estradiol synthesis and hence increase their reproductive efficiency.
Keywords: Aspirin, osmotic fragility, steroidal hormones, laying chickens.

INTRODUCTION
Osmotic fragility is the tendency of the red blood cells to lyse in hypotonic medium. This physiological phenomenon is often used to determine the health status or predict underlying predisposing factors to ailments or health challenges in both livestock and humans (Aro *et al.*, 2010; Aro and Akinmoyegun, 2012).

Acetyl salicylic acid (ASA) is a known antipyretic and analgesic agent. Its use as a dietary supplement to reduce heat stress and improve production performance in livestock is widely known. Little information is however available on its effect on the osmotic fragility of the red blood corpuscles and circulating pituitary and steroidal hormones especially in laying chickens. This experiment was therefore conducted to investigate the responses of two commercial breeds of layers- the Isa Brown and Harco Black to dietary inclusion of ASA using the osmotic fragility of their red blood cells and circulating estradiol, testosterone, progesterone and cortisol as response criteria

MATERIALS AND METHODS
Experimental site: This research work was carried out at the Teaching and Research Farm (Livestock unit) of the Federal University of

Technology, Akure. The farm is located in the humid rain forest zone of western Nigeria which is characterized by two rainfall peaks and high humidity during the rainy season. The mean annual rainfall is about 1,500mm and rain lasts for 9 months usually from March to November every year. The mean annual relative humidity is over 75% and that of temperature is about 27°C.

Experimental Materials: One hundred and ninety two point of lay birds were used i.e. 96 Harco Black and 96 Isa Brown point of lay birds were purchased from reputable Farms in Ibadan, Oyo .State Nigeria. Four experimental diets with varying levels of acetylsalicylic acid (ASA) were formulated. The first diet was the control with 0.00% of ASA and labeled as T1 while the remaining three (3) diets were labeled as T2, T3 and T4 with 0.025kg, 0.050kg and 0.075kg of ASA per 100kg of feed respectively. Birds were randomly allotted into four treatments (diets) which contained 48 birds each. Each treatment was replicated four times with twelve (12) birds per replicate. The feeding trial lasted for 16 weeks.

Managements Practices: The birds were fed *ad-libitum* for 12hours daylight period.Medications and vaccinations were administered as at when due and spent litter materials were removed every week.

Data Collection and Analysis: Blood samples were collected at the end of 16th week of the experiments through venipuncture of the axillary vein of each bird into the Ethylene Diamene Tetra Acetic Acid (EDTA) bottles and sterile test tubes concurrently. The blood samples in the EDTA bottles were used to test for osmotic fragility as described by Oyewale (1991) while those in the sterile test tubes were slanted and allowed to clot after which the sera were harvested and use for hormonal assay. Progesterone, testosterone E2 and cortisol were assayed using Tosoh Bioscience protocols.Data collected were subjected to completely randomized design and two way analysis of variance using SAS 2003 version 9.2 statistical package. Significant differences where appropriate were determined using Duncan multiple range test (DMRT) of the same statistical package.

RESULTS AND DISCUSSION

Table 1 shows the percentage of red blood cells haemolysed per saline concentration among the treatments, between the breeds and their interactions. Results from blood sample collected at the end of the experiment showed significant ($P<0.05$) differences in the erythrocytic fragility between the IB and HB breeds. IB was more osmotically stable to erythrocytic fragility than the HB breed at all saline concentrations.This indicated that IB breed has an adaptive advantage for survival in the tropics compared to HB. This wassimilar to the report from Habibu *et al.* (2013), who reported that Marshal breed erythrocytes appeared to be osmotically more resistant then the Anak-Hubbard cross, while Ross breed had the highest fragility. Breed difference in erythrocyte osmotic fragility as observed in this study is in agreement with other previous studies in birds (Oyewale and Durotoye, 1988). The interaction between the breed and the treatment showed an increase in osmotic stability of red blood cells (RBC) of the HB with increase in ASA level while IB breed showed a decrease in osmotic stability of RBC with increase in the levels of ASA in the diets. It therefore meant that the IB breed would be more osmotically liable to lysis at higher ASA concentration than the HB breed.

Table 2 showed the effect of dietary ASA on circulating pituitary and steroidal hormones. Results revealed that there were breed differences in E2 and testosterone concentration with the HB recording a higher ($p<0.05$) E2 (440.38±92.62pmol/L) versus (229.37±84.10pmol/L) and testosterone (0.68±0.11nmol/L versus 0.12±0.08nmol/L) than the IB breed. There were significant treatment effects of ASA on progesterone, testosterone and E2 concentration in which these three parameters showed gradual increase with increase in dietary levels of ASA. This report is not in agreement with Al-Bayaty (2002) and Oyedeji*et al.* (2013) who reported that aspirin caused significant decrease in testosterone levels in albino rats. The effects of breed versus treatment revealed that all the four parameters –progesterone, testosterone, E2 and cortisol differed significantly. The HB breed however gave a more consistent increase in the circulating serum values for all these parameters than in the IB breed.

CONCLUSION

It can be concluded that Isa Brown breed was more fragile and osmotically liable to lysis at higher dietary ASA concentration than the Harco Black breed. Increase in dietary ASA level caused gradual increase in progesterone, testosterone and E2 concentration in the laying chickens.

REFERENCES

Al Bayati, S. A. (2002). The effect of aspirin on female reproductive system in mice. A thesis submitted to the college of medicine, university of Baghdad. Pp 1423.

Aro, S. O. and Akinmoyegun, M. B. (2012).Haematology and Red Blood Cells Osmotic Fragility of Pigs Fed Graded Levels of Fermented Cassava Peels Based Diet. *Proceedings of 17th Annually Conference of Animal Science Association of Nigeria (ASAN),* 9th – 15th September, 2012. International Conference Center, Area 8, Buja, Nigeria.Pp 152 -155.

Aro, S. O., Adejumo, D. O., Aleeto, V. A. and Agbole, E.H. (2010). Osmotic Fragility of Pocine Erythrocytes Fed Fermented Cassava Tuber Waste Based Diet. *15th ASAN Annual Conference*, ASANUYO, 2010, 13/15 September, 2010. Pp 209-212

Habibu, B., Yaqub, L. S., Ahmed, I. A.

Kawu, M. U., Buhari, H.U., Tauheed, M. and Isa, H.I. (2013).Erythrocyte Osmotic Fragility and Haematologic Parameters of Three Breeds of 9-Week-Old Broiler Chickens.*International Journal of Poultry Science,* 12 (5): 277-279.

Mohamed, A. E., Abdel Rahman, M., Hereba, M. and Shawki M. (2012).Effects of Aspirin on Rheological Properties of Erythrocytes *InVitr.Int J Biomed Sci*; 8 (3): 188-193)

Oyedeji, K.O., Bolarinwa, A. F and Adigun, A. K. (2013).Effect of Aspirin on Reproductive Functions in Male Albino Rats.*Journal of Pharmacy and Biological Sciences;* Volume 4, PP 49-54

Oyewale, J. O. (1991). Osmotic fragility of erythrocyte of guinea-fowl at 21 and 156 weeks of age.*VeterinarskiArhiv*; 61(1) 49 – 56

Oyewale, J.O. and Durotoye, L.A. (1988).Osmotic fragility of erythrocytes of two breeds of domestic fowl in the warm humid tropic.*Lab. Anim.*, 22: 250-254

Table 1: Number of Red Blood Cells (x^6) that are Osmotically Stable per Saline Concentration.

SOV	ASA Level (%)	0% NaCl	0.1% NaCl	0.2% NaCl	0.3% NaCl	0.4%NaCl	0.5%NaCl	0.6%NaCl	0.7%NaCl	0.8%NaCl	0.9% NaCl	SEM
Breed												
IB		1.24	1.42[a]	1.55[a]	1.71[a]	1.76[a]	1.88[a]	2.01[a]	2.15[a]	2.28[a]	2.36[a]	0.13
HB		0.92	1.05[b]	1.17[b]	1.29[b]	1.41[a]	1.54[b]	1.65[b]	1.76[b]	1.84[b]	1.98[b]	0.09
T												
T1	0.00	1.24	1.41	1.52	1.63	1.74	1.86	2.01	2.24	2.27	2.37	0.22
T2	0.025	0.95	1.08	1.18	1.32	1.45	1.59	1.70	1.82	1.87	2.02	0.17
T3	0.050	0.99	1.12	1.24	1.44	1.49	1.61	1.73	1.76	1.98	2.07	0.15
T4	0.075	1.19	1.39	1.55	1.66	1.71	1.83	1.94	2.06	2.19	2.27	0.16
BxT												
IB	0.00	1.58	1.74	1.85	1.95	2.08	2.21	2.36	2.68	2.64	2.74	0.30
HB	0.00	0.74	0.92	1.03	1.15	1.23	1.33	1.49	1.59	1.71	1.82	0.04
IB	0.025	1.02	1.18	1.29	1.44	1.55	1.69	1.80	1.93	2.06	2.12	0.27
HB	0.025	0.89	0.98	1.07	1.21	1.34	1.49	1.60	1.71	1.67	1.91	0.22
IB	0.050	1.12	1.25	1.38	1.64	1.66	1.77	1.89	1.85	2.16	2.23	0.22
HB	0.050	0.86	0.99	1.10	1.24	1.33	1.46	1.58	1.67	1.80	1.91	0.21
IB	0.075	1.25	1.54	1.70	1.82	1.76	1.86	1.99	2.12	2.25	2.33	0.27
HB	0.075	1.14	1.27	1.42	1.53	1.67	1.80	1.89	2.01	2.15	2.22	0.20
B		NS	*	*	*	*	*	*	*	*	*	
T		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	
B*T		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	

a, b =means on the same row but with different superscripts are statistically ($p<0.05$) significant. SOV= Source of Variation; B= Breed; T= Treatment; IB = Isa Brown: HB = Harco Black. SEM = Standard error of the mean.

Table 2: Hormonal Indices of the Laying Hens fed Varying Levels of Acetyl salicylic acid (ASA)

SOV	ASA Level (%)	LH (IU/L)	PROG (nmol/L)	TEST (nmol/L)	E2 (pmol/L)	CORTISOL (nmol/L)
Breed						
Isa Brown		0.20±0.00	0.70±0.12	0.12±0.08^{b}	229.37±84.10^{b}	1.35±0.83
Harco Black		0.20±0.00	0.73±0.07	0.68±0.11^{a}	440.38±92.62^{a}	2.18±1.05
T						
T1	0.00	0.20±0.00	0.53±0.05^{c}	0.43±0.03^{b}	91.80±0.00^{b}	2.18±1.67
T2	0.025	0.20±0.00	0.63±0.08^{b}	0.64±0.17^{a}	333.81±140.07^{a}	0.52±0.00
T3	0.050	0.20±0.00	0.86±0.20^{a}	0.66±0.17^{a}	438.12±124.40^{a}	2.18±1.67
T4	0.075	0.20±0.00	0.84±0.10^{a}	0.80±0.16^{a}	475.75±143.63^{a}	2.18±1.67
BXT						
Isa Brown	0.00	0.20±0.00	0.53±0.08^{b}	0.47±0.01^{b}	91.80±0.00^{c}	3.02±2.50^{b}
Harco Black	0.00	0.20±0.00	0.52±0.00^{b}	0.37±0.00^{b}	91.80±0.00^{c}	0.52±0.00^{b}
Isa Brown	0.025	0.20±0.00	0.53±0.00^{b}	0.30±0.00^{c}	91.80±0.00^{c}	0.52±0.00^{b}
Harco Black	0.025	0.20±0.00	0.68±0.10^{a}	0.81±0.04^{a}	454.82±122.19^{a}	0.52±0.00^{b}
Isa Brown	0.05	0.20±0.00	0.89±0.35^{a}	0.72±0.23^{a}	409.27±209.59^{a}	0.52±0.00^{b}
Harco Black	0.05	0.20±0.00	0.82±0.00^{a}	0.52±0.00^{a}	495.83±0.00^{a}	5.52±0.00^{a}
Isa Brown	0.075	0.20±0.00	0.86±0.00^{a}	0.82±0.00^{a}	282.26±0.00^{b}	0.52±0.00^{b}
Harco Black	0.075	0.20±0.00	0.88±0.35^{a}	0.79±0.28^{a}	572.50±183.86^{a}	3.02±2.50^{b}
Breed		NS	NS	*	*	NS
Treatment		NS	*	*	*	NS
Breed*Treatment		NS	*	NS	*	*

a, b, c =means on the same row but with different superscripts are statistically ($p<0.05$) significant. Each value represents the mean for 3 birds selected at random per treatment; E2 = Estradiol; LH = Luteinizing Hormone; PROG = Progesterone and TEST = Testosterone. ASA: Acetyl salicylic acid.
T1 = Diet with 0.00% ASA; T2 = Diet with 0.025% ASA; T3 = Diet with 0.50% ASA; T4 = Diet with 0.075% ASA; NS = Not significant; SOV= Source of Variation; B= Breed; T= Treatment; * = Significant at 0.05%.