AMM -07

Blood Viscosity and Osmotic Fragility of Two Breeds of Chickens Fed Dietary Supplementation of Acetylsalicylic Acid (ASA)

S.O. Aro, O.B. Faluyi, J.O. Arogbodo and O.G. Taylor
Department of Animal Production and Health, Federal University of Technology, Akure, Nigeria.
Corresponding author: S.O. Aro; E-mail: soaro@futa.edu.ngPhone No: +2347062329254

Abstract
This experiment was performed to determine the effect of feeding varying levels of dietary acetylsalicylic acid (ASA) on the osmotic fragility and blood viscosity of two strains of layers. Four experimental diets designated as: T1 (control diet with 0.0% ASA), T2 (diet with 0.25% ASA), T3 (diet with 0.50% ASA) and T4 (diet with 0.75% ASA) were fed to one hundred and ninety two layers of two different breeds - Harco Black (HB) and Isa Brown (IB) layers which were grouped into forty-eight birds per treatment, replicated four times at twelve birds per replicate. Blood samples were collected and analyzed at the end of the experiment to ascertain the effect of dietary acetylsalicylic acid (ASA) on red blood cell osmotic stability and viscosity of whole blood, serum and plasma. The result on osmotic fragility showed that there were significant differences ($p<0.05$) between the two breeds of layers, among the four treatments and in the interaction between the breeds and the treatments. Isa Brown layers were more osmotically stable than Harco Black layers at all levels of saline concentrations. Also, 0.075% ASA supplementation significantly improved the red blood osmotic stability beyond 0.30% saline concentration. The blood thinning effect of ASA was most beneficial at 0.050% level. It can be concluded that increase in the dietary ASA up to 0.75% level had significant effect on the two breeds of commercial breeding layers as far as their red blood cell stability is concerned.

Keywords: Blood cells, Harco Black, Isa Brown, osmotic stability, blood viscosity

Introduction

The effects of climate change with its variants of climatic phenomena like the *El Nina* and *El Nino* have unleashed a plethora of climatic variability on the global ecological belts with untoward effects on both human and livestock population. This is more impactful within the Tropics known for its generally high ambient temperature and radiant heat that subject livestock within the region to environmental stressors. Several mitigating strategies have been advanced towards the problems of heat stress in particular as it affects livestock welfare and productivity in tropical countries like the use of ascobates, chlorides of potassium and sodium(Aro and Akinleminu, 2015) and even vitamin E. With the seemingly relentless scourge of climate change on the welfare and productivity of the livestock enterprise, further search into addressing this scourge has been sought for example through the use of acetyl salicylic acid (Aspirin) in the diets of poultry birds to combat heat stress (Aro *et al.,* 2017).

Aspirin (acetylsalicylic acid) is a known anti-pyretic and anti-inflammatory agent. It is also reputed as a blood thinner because of its inhibitory role on prostaglandin synthesis in blood platelets. Aspirin treatment has also been found to decrease free radical stress, through decreased lipid peroxidation and the maintenance of glutathione content (Bode-Böger *et al.,* 2005). A lot of research works have been carried out on the use of ASA as anti-stress, anti-pyretic and anti-inflammatory drugs. But little work has been done on its effects on the blood viscosity and osmotic fragility.

This research was therefore designed to look at the effects of ASA on performance of birds at the levels of the blood viscosity and osmotic fragility.

Materials and Method

This research work was carried out at the Teaching and Research Farm (Livestock unit) of the Federal University of Technology, Akure. 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. Table 1 shows the gross composition of the experimental diets.

One hundred and ninety two layers comprising ninety six each of Harco Black and Isa Brown layers were randomly allotted to the four treatment diets at 48 birds each. Each treatment was replicated four times with twelve birds per replicate. At the end of the experiment, blood samples from forty eight birds (12 birds per treatment group) were collected through the jugular vein of each bird for the determination of osmotic fragility and

Table 2: Osmotic stability of the red blood cells of layers fed varying supplemental levels of Acetylsalicylic acid.

blood viscosity. The values of the whole blood, serum and plasma viscosity were calculated from the values obtained from the viscometer reading using the formula below;

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

Where 1.0038 is the viscosity of water at standard temperature and pressure and the flow time of water is 2.74 seconds. Erythrocyte osmotic fragility (EOF) test was carried out using NaCl and distilled water.

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

Ingredients	T1 (kg)	T2 (kg)	T3 (kg)	T4 (kg)
Maize	50.00	50.00	50.00	50.00
Soybean Meal	12.00	12.00	12.00	12.00
Wheat Offal	17.00	17.00	17.00	17.00
Groundnut cake	6.50	6.50	6.50	6.50
Palm kernel cake	3.50	3.50	3.50	3.50
Fishmeal	1.00	1.00	1.00	1.00
Bone meal	2.60	2.60	2.60	2.60
Limestone	6.50	6.50	6.50	6.50
Methionine	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Layer's premix	0.25	0.25	0.25	0.25
Salt	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00
ASA	0.00	0.025	0.050	0.075

T1: Control diet with 0.00% ASA; T2: Diet with 0.025% ASA; T3: Diet with 0.050% ASA; and T4: Diet with 0.075% ASA; ASA = Acetylsalicylic acid; ME = Metabolizable Energy.

Resultsand Discussion

Table 2 shows the result number of red blood cells ($x10^6mm^{-3}$) that are osmotically stable at various saline concentrations. The result revealed significant ($p<0.05$) breed differences in all saline concentrations between the Isa Brown (IB) and the Harco Black (HB) breeds in which the IB was the more osmotically stable breed. This supports the breed difference in osmotic stability between the Marshall breed and Anak-Hubbard cross reported Habibu *et al.* (2013). The results revealed that the use of ASA at 0.075% conferred the best osmotic stability on the red blood cell membranes of the birds. Also, the HB breed was more sensitive to the ameliorative action of ASA on erythrocytic stability than the IB breed.Table 3 shows whole blood, serum and plasma viscosities of Isa Brown and Harco Black breeds of layers fed varying levels of ASA supplementation. There were no significant breed differences ($p>0.05$) in whole blood, plasma and serum viscosities but significant treatment effects as well as interaction between breed and treatments were observed. The general trend was an increase in viscosity with increase in ASA supplementation.

Conclusion and Recommendations

Dietary supplementation of diets of layers with ASA could ensure better stability of the red blood cell membranes under hypotonic pressure. The IB breed seemed the more osmotically stable breed than the HB breed but was less sensitive than the latter breed to the ameliorative action of ASA the osmotic stability of the red blood cell membrane. Further experiment should be performed to establish the effect of ASA on blood viscosity because the current study negates its popular belief as a blood thinner.

Treatments	Breed	0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
	HB	1.89±0.14^{b}	1.92±0.14^{b}	1.96±0.14	2.01±0.14^{b}	2.06±0.15^{b}	2.11±0.15^{b}	2.16±0.15^{b}	2.21±0.15^{b}	2.27±0.15^{b}	2.46±0.16^{b}
	IB	2.08±0.12^{a}	2.11±0.12^{a}	2.15±0.12	2.20±0.12^{a}	2.25±0.12^{a}	2.29±0.12^{a}	2.32±0.12^{a}	2.40±0.12^{a}	2.45±0.12^{a}	2.61±0.13^{a}
1		1.98±0.13^{a}	2.02±0.13^{a}	2.05±0.13^{a}	2.09±0.13^{a}	2.14±0.13^{b}	2.18±0.13^{b}	2.23±0.14^{b}	2.27±0.14^{b}	2.32±0.14^{b}	2.46±0.15^{b}
2		1.95±0.14^{a}	1.98±0.14^{a}	2.01±0.14^{b}	2.06±0.14^{b}	2.11±0.14^{b}	2.16±0.13^{b}	2.21±0.13^{b}	2.26±0.13^{b}	2.31±0.13^{b}	2.49±0.13^{b}
3		1.87±0.27^{b}	1.90±0.27^{b}	1.94±0.27^{b}	1.99±0.27^{b}	2.03±0.28^{b}	2.08±0.28^{b}	2.09±0.27^{b}	2.17±0.28^{b}	2.22±0.29^{b}	2.41±0.30^{b}
4		2.14±0.19^{a}	2.17±0.19^{a}	2.22±0.19^{a}	2.28±0.19^{a}	2.34±0.19^{a}	2.40±0.19^{a}	2.46±0.19^{a}	2.52±0.19^{a}	2.58±0.20^{a}	2.77±0.20^{a}
1	HB	2.19±0.16^{a}	2.22±0.16^{a}	2.26±0.16^{a}	2.30±0.16^{a}	2.35±0.16^{a}	2.41±0.16^{a}	2.46±0.17^{a}	2.50±0.17^{a}	2.56±0.17^{a}	2.73±0.18^{a}
1	IB	1.78±0.16^{b}	1.81±0.17^{b}	1.84±0.17^{b}	1.88±0.17^{b}	1.92±0.17^{b}	1.95±0.18^{b}	2.00±0.18^{c}	2.05±0.18^{b}	2.09±0.18^{b}	2.19±0.19^{c}
2	HB	1.84±0.13^{b}	1.87±0.13^{b}	1.90±0.12^{b}	1.95±0.13^{b}	2.00±0.13^{b}	2.05±0.13^{b}	2.11±0.13^{b}	2.16±0.12^{b}	2.21±0.13^{b}	2.40±0.10^{b}
2	IB	2.06±0.25^{a}	2.09±0.25^{a}	2.12±0.25^{a}	2.16±0.25^{a}	2.22±0.24^{a}	2.26±0.24^{a}	2.31±0.24^{b}	2.36±0.24^{c}	2.41±0.24^{a}	2.57±0.24^{a}
3	HB	1.45±0.36^{c}	1.49±0.37^{c}	1.53±0.37^{c}	1.57±0.38^{c}	1.61±0.39^{c}	1.66±0.39^{c}	1.70±0.40^{d}	1.74±0.40^{c}	1.78±0.41^{c}	1.97±0.45^{d}
3	IB	2.30±0.34^{a}	2.31±0.33^{a}	2.36±0.33^{a}	2.41±0.34^{a}	2.45±0.34^{a}	2.49±0.34^{a}	2.48±0.34^{a}	2.60±0.34^{a}	2.65±0.34^{a}	2.84±0.35^{a}
4	HB	2.08±0.36^{a}	2.11±0.36^{a}	2.16±0.36^{a}	2.22±0.36^{a}	2.28±0.36^{a}	2.34±0.37^{a}	2.40±0.37^{a}	2.46±0.37^{a}	2.52±0.37^{a}	2.72±0.38^{a}
4	IB	2.20±0.17^{a}	2.23±0.17^{a}	2.28±0.17^{a}	2.35±0.17^{a}	2.41±0.16^{a}	2.46±0.16^{a}	2.52±0.16^{a}	2.58±0.17^{a}	2.64±0.17^{a}	2.82±0.17^{a}
Breeds		*	*	*	*	*	*	*	*	*	*
Treatment		*	*	*	*	*	*	*	*	*	*
Breeds*Treatment		*	*	*	*	*	*	*	*	*	*

a,b,c,d = Means in the same column bujt with different superscripts are statiscally (P<0.05) significant.
1 = Diet with 0.00 % ASA; 2 = Diet with 0.025 % ASA; 3 = Diet with 0.050 % ASA; 4 = 0.075 %ASA; HB = Harco Black; IB = Isa Brown; B = Breeds; T = Treatments; BxT = Breed Versus Treatment; ASA = Acetylsalicylic acid.

Table 3: Whole Blood, plasma and serum viscosity of Harco Black and Isa Brown layers fed different levels of dietary ASA supplementation.

Treatment1	Breed	W.blood Viscosity	Plasma Viscosity	Serum Viscosity
	HB	2.42±0.05	1.35±0.08	1.34±0.07
	IB	2.44±0.11	1.32±0.08	1.32±0.09
1		2.27±0.12^{c}	1.27±0.09^{ab}	1.22±0.09^{b}
2		2.28±0.04^{c}	1.16±0.08^{b}	1.24±0.01^{b}
3		2.54±0.10^{b}	1.34±0.08^{ab}	1.44±0.15^{a}
4		2.63±0.07^{a}	1.58±0.10^{a}	1.42±0.14^{a}
1	HB	2.45±0.08^{c}	1.35±0.06^{c}	1.17±0.09^{c}
1	IB	2.10±0.16^{f}	1.19±0.19^{d}	1.27±0.19^{c}
2	HB	2.31±0.06^{e}	1.06±0.04^{e}	1.25±0.02^{c}
2	IB	2.26±0.07	1.27±0.13^{d}	1.23±0.03^{c}
3	HB	2.36±0.03^{d}	1.41±0.02^{b}	1.34±0.05^{b}
3	IB	2.72±0.03^{a}	1.28±0.17^{d}	1.55±0.34^{a}
4	HB	2.58±0.12^{b}	1.60±0.17^{a}	1.60±0.17^{a}
4	IB	2.68±0.10^{b}	1.57±0.19^{a}	1.23±0.12^{c}
B		NS	NS	NS
T		*	*	*
B xT		*	*	*

a,ab,b, c, d, e, f = means on the same column but with different superscripts are statistically (p<0.05) significant. 1 = Diet with 0.00% ASA; 2 = Diet with 0.025% ASA; 3 = Diet with 0.050% ASA; 4 = Diet with 0.075% ASA; HB =Harco Black; IB = Isa Brown; NS = Not significant; Sig =Significant; * = Significant at 0.05%. B= Breed; T= Treatment.

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