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Haemogram, blood viscosity and osmotic fragility of Isa White and Barred Plymouth Rock cocks fed varying levels of salt

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SUMMARY

This experiment was performed to determine the effect of diets with varying salt levels on the haemogram, whole blood viscosity and osmotic fragility of Barred Plymouth Rock (BPR) and Isa White (IW) breeding cocks. Four experimental diets designated as: T1 (control diet with 0.25 % salt), T2 (diet with 0.50 % salt), T3 (diet with 0.75 % salt) and T4 (diet with 1.00 % salt) were fed to 48 cocks of the two different breeds grouped into 12 birds per treatment and at four birds per replicate. At the end of eight weeks of experiment, blood samples were collected for the determination of the haemogram, whole blood viscosity and red blood cell osmotic fragility. The results revealed differences (P<0.05) in erythrocyte sedimentation rate, haemoglobin content, red blood corpuscles, packed cell volume and whole blood viscosity between the two breeds. Also, percentage eosinophils (3.30±0.15 versus 3.10±0.10) and basophils (1.60±0.16 versus 1.80±0.13) differed (P<0.05) between breeds. The red blood cells of birds on the high salt diets were more osmotically fragile than the control diet. There was no breed difference in the osmotic fragility between the BPR and IW cocks, however the BPR breed was more sensitive to osmotic fragility at all saline concentrations and dietary levels than the IW breed. Conclusively, increase in the dietary salt up to 0.75 % level has no significant effect on the two commercial breeding cocks as far as their differential leucocyte count is concerned, but a case of sub-clinical basophilia could be induced at 1.00% level. Also, the IW seemed the more osmotically stable breed in terms of its erythrocytic osmotic fragility.

Additional keywords

Breeder cocks.
Chickens.
Erythrocytes.
Leucocytes.
Osmotic fragility.

Hemograma, viscosidad de la sangre y fragilidad osmótica de gallos Isa White y Plymouth Rock barrado alimentan con diferentes niveles de sal

RESUMEN

Este experimento fue realizado para determinar el efecto de dietas con diferentes niveles de sal en el hemograma, viscosidad de la sangre y la fragilidad osmótica de Plymouth Rock barrado (BPR) y Isa blanco (IW) pollas de cría. Cuatro dietas experimentales señaladas como: T1 (control de dieta con 0.25% de sal), T2 (dieta con 0.50% de sal), T3 (dieta con 0.75% de sal) y T4 (dieta con sal 1.00%) fueron alimentados a 48 pollas de las dos razas diferentes, agrupadas en 12 aves por tratamiento y en cuatro aves por repetición. Al final de ocho semanas del experimento, se recogieron muestras de sangre para la determinación del hemograma, viscosidad de la sangre y la fragilidad osmótica del glóbulo rojo. Los resultados revelaron diferencias (P < 0.05) en la tasa de sedimentación eritrocítica, contenido de hemoglobina, rojo sangre corpúsculos, embalado de la célula volumen y sangre entera de la viscosidad entre las dos razas. También, el porcentaje de eosinófilos (3.30±0.15 versus 3.10±0.10) y basófilos (1.60±0.16 versus 1.80±0.13) difieren (P < 0.05) entre las razas. Los glóbulos rojos de aves en las dietas altas en sal eran más osmóticamente frágiles que la dieta control. No hubo diferencias de raza en la fragilidad osmótica entre el BPR y IW pollas, sin embargo la raza BPR fue más sensible a la osmótica fragilidad en todos los niveles dietéticos y concentraciones salinas que la raza de IW. Concluyente, aumento en nivel de 0.75% hasta sal dietética no tiene ningún efecto significativo sobre las dos pollas de cría comercial en cuanto se refiere a su diferencial leucocitaria, pero un caso del basophilia subclínica podía ser inducido a nivel de 1.00%. También, el IW parecía la raza más osmóticamente estable en cuanto a su fragilidad osmótica eritrocitaria.

Palabras clave adicionales

Criador de gallos.
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INTRODUCTION

The role of dietary salt or sodium chloride (NaCl) is multifaceted. It is a known appetiser in foods and feeds as it is a yardstick for measuring voluntary feed intake. Palatability is also measured by the direct feel of saltiness by the palate. Salt plays the physiological role as the harbinger of the two major electrolytes of the blood – the sodium and chloride ions. These sodium (Na^+) and chloride (Cl^-) ions are monovalent and hence confer greater effects on the acid-base balance of body than the divalent or polyvalent ions because of their stronger ionising properties. Increased blood concentration of the two ions could therefore predispose the body to alkalosis or acidosis respectively (Mushtaq & Pasha 2013). A balance therefore needs to be struck between the deficiency of NaCl on one hand and its excessive use in the diets on the other hand. For instance, its deficiency in human diet (Johnson 2007; Morris, Na & Johanson 2008) and in cattle (Phillips et al. 1999) has been reported to provoke spontaneous physiological adjustment and profound behavioural changes. Its deficiency in poultry has been linked to loss of appetite and weight (Hagsten & Perry 1976), anxiety and vice habits like feather and vent pecking. On the other hand, excessive sodium chloride intake could adversely affect livestock performance. Aro (2014) reported that increase in salt content of diets could lead to increase in the core body temperature of pigs which could aggravate thermal stress in the tropical environment. Also, Cowieson et al. (2011) observed that increased dietary sodium chloride concentrations reduce endogenous amino acid flow and influence the physiological response to the ingestion of phytic acid by broiler chickens. Dietary salt has also been shown to confer immunity on rats (http://news.sciencemag.org/biology/2015) hence diets that are grossly deficient in NaCl could result in unthriftiness and heavy mortality in livestock population. Dietary salt therefore improves livestock livability by potentiating their immuno-competence.

There is however breed differences in utilization and tolerance to dietary NaCl among livestock population. For example, Merck Veterinary Manual (2015) reported that susceptibility to high salt intake is more common in swine (the most sensitive species), cattle, and poultry while ruminants like sheep and goats are relatively resistant, thus putting the acute oral lethal dose of salt as 2.2 g/kg in swine and 6.0 g/kg in sheep. To my knowledge breed differences in salt tolerance especially in the popular Isa White and Barred Plymouth Rock breeder cocks have not been investigated. Since the immuno-competence and livability of stock lie on the white blood cells and the functionality of its other cellular and non-cellular components like the red blood cells, platelets and the electrolytes like sodium, potassium and chloride ions; hence using these blood components as markers of response to dietary salt intake would be very appropriate. This study therefore chose to investigate the effects of varying levels of dietary salt on the haemogram, differential white blood cell counts and osmotic fragility of the red blood cells of Barred Plymouth Rock and Isa White cocks.

Table I. Gross composition (g/100g) of the experimental diets (Composición bruta (g / 100g) de las dietas experimentales).

Ingredients	T1	T2	T3	T4
Maize	54.30	54.30	54.30	54.30
Wheatoffal	2100	20.75	20.50	20.25
Groundnutcake	9.00	9.00	9.00	9.00
Soyabeanmeal	9.00	9.00	9.00	9.00
Bone meal	3.00	3.00	3.00	3.00
Limestone	1.50	1.50	1.50	1.50
Fishmeal	1.00	1.00	1.00	1.00
Salt	0.25	0.50	0.75	1.00
Layers' Premix*	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00
Calculated analysis				
CrudeProtein (%)	16.80	16.78	16.76	16.74
M.E = (KJ/kg)	10.89	10.89	10.89	10.89
CrudeFibre (%)	4.51	4.51	4.51	4.51
Fat and Oil (%)	4.47	4.47	4.47	4.47
Calcium (%)	1.62	1.62	1.62	1.62
Phosphorus (%)	1.00	1.00	1.00	1.00
Lysine (%)	0.85	0.85	0.85	0.85
Methionine (%)	0.37	0.37	0.37	0.37

*Layers'premix: Supplied the following /kg of feed: Vitamin A, 10,000 iu; vitamin D, 2,000 iu; vitamin B_1, 0.75mg; nicotinic acid, 25mg; calcium pantothenate, 12.50mg; vitmin B_{12}, 2.5mg; vitamin K, 2.5mg; vitamin E, 2.5mg; cobalt, 0.40mg; biotin, 0.50mg; folic acid, 10mg; choline chloride, 25mg; copper, 8mg; manganese, 64mg; iron, 32mg; zinc, 40mg; iodine,0.8mg; L-lysine, 120mg; DL-methionine, 50mg; selenium, 0.16mg; T1= Diet with 0.25% salt; T2= Diet with 0.50 % salt; T3= Diet with 0.75 % salt; T4= Diet with 1.00 % salt.

MATERIALS AND METHODS

The experiment was carried out at the poultry unit of the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria. Akure (Latitude 7° 18′ N and Longitude 5° 10′ E) is located in the humid rain forest zone of western Nigeria which is characterized by two rainfall peaks and high humidity during the raining season. The mean annual rain fall is about 1500 mm and the rains last for about nine months usually March to November of every year. The mean annual relative humidity is over 75 % while the mean annual temperature is about 27°C.

EXPERIMENTAL ANIMALS

Permission to use the experimental animal was sought and obtained from the Research and Ethics Committee of the Federal University of Technology, Akure, Nigeria. A total of twenty-four (24) Isa White and twenty-four (24) Barred Plymouth Rock cocks of about 10 months of age were purchased from a reputable source and used for the experiment. The birds were acclimatized for a period of two weeks after which the eight-week feeding trial commenced. The cocks were randomly allotted to four (4) dietary treatments with twelve (12) cocks in each treatment. Each treatment was replicated thrice at four birds per replicate. They were housed in a battery cage and supplied with feed and water *ad libitum* throughout the period of the experiment. Four experimental diets with varying levels of dietary salt were formulated. The first diet which had 0.25 kg of salt/100 kg of feed served as the control diet (T1). The remaining diets were labelled T2 with 0.50 kg of salt/100 kg of feed, T3 with 0.75 kg of salt/100 kg of feed and T4 with 1.00 kg of salt/100 kg of feed respectively **(Table I)**.

DATA COLLECTION

At the end of the 8th week of the experiment, all the twelve (12) birds from each treatment were bled through jugular vein puncture. Blood meant for the determination of haematological indices, osmotic fragility and whole blood viscosity were collected into sample bottles coated with Ethylene-Diamine Tetra-Acetic Acid (EDTA). The blood samples were slightly agitated to avoid coagulation. Haematological parameters determined were haemoglobin (Hb) content, red blood cell (RBC) count, white blood cell(WBC) count and the differential WBC count. The packed cell volume (PCV) was determined by centrifuging the micro haematocrit capillary tubes containing blood samples in a haematocrit centrifuge for 5 minutes at 3,000rpm and after which values were read with haematocrit reader. The RBC, WBC and the differential counts were determined using improved Neubauer haemocytometer, while the Hb was determined using cyanmethaemoglobin method of Jain (1986).

The osmotic fragility was determined as previously described by Oyewale (1991) using NaCl and distilled water. Briefly, ten bottles were each filled with 100ml of distilled water after which 0.00g to 0.90g of NaCl was measured and dissolved respectively into each of the bottle to give a saline concentration that ranged from 0.00 % to 0.90 %. Measured quantity (0.05 millilitre) of the blood collected from the animals was added to the saline solution in the test tubes from which the percentage of the red blood cells haemolysed per saline concentration was calculated and used as a measure of red blood cell osmotic fragility. The whole blood viscosity was determined with the use of Oswald viscometer and a stop watch. The values of the whole blood 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 2.85 seconds.

STATISTICAL ANALYSIS

All data were subjected to a 2 x 4 factorial analysis in a completely randomized design using SAS (2008) statistical package. Mean separation where applicable was done with Duncan's multiple range test of the same statistical package and significance was accepted at 5.00% level.

RESULTS

Table II shows the haemogram and whole blood viscosity of Barred Plymouth Rock and Isa White cocks fed varying levels of dietary salt. There were breed differences (P<0.05) in erythrocyte sedimentation rate (ERS), haemoglobin (Hb) content, red blood corpuscles (RBC),packed cell volume (PCV) and whole blood viscosity (WBV) between the Isa White (IW) and Barred Plymouth Rock (BPR) cocks. The IW cocks had higher ESR but lower Hb, RBC, PCV and WBV than the BPR cocks. There were also significant treatment effects in all the haematological parameters considered in **Table II**. The ESR, WBV, MCV and MCH decreased relative to the control while Hb and RBC increased. Also the increase or decrease observed in these blood parameters was concomitant with increase in dietary salt levels. For instance, the ESR decreased from 1.80±0.37mm/hr in T1 to 1.20±0.20mm/hr in T4 while the RBC increased from $4.31\pm0.51\times10^{6}mm^{-3}$ in T1 to $5.36\pm0.60\times10^{6}mm^{-3}$ in T4. The interaction between breeds and treatments followed the same trend observed under the responses of the blood parameters to varying dietary salt levels. For example, the MCV decreased in the BPR breed from 77.44±6.87fl in T1 to 71.45±2.65fl in T4 while it

Table II. The haemogram and whole blood viscosity of Barred Plymouth Rock and Isa White Cocks fed varying levels of dietary salt (La viscosidadhemograma y sangreentera de la roca de Plymouth barrado y Isa blancopollasalimentados con diferentesniveles de saldietética).

Treatments	Breed	ESR (mm/hr)	PCV (%)	RBC ($\times10^{6}mm^{3}$)	HB (g/dl)	WBV	MCV (fl)	MCHC (%)	MCH (pg)
	BPR	1.30 ± 0.15^{b}	37.40 ± 1.34^{a}	5.04 ± 0.37^{a}	12.61 ± 0.42^{a}	5.09 ± 0.18^{a}	76.75±4.01	33.76±0.39	25.97±1.51
	IW	1.70 ± 0.21^{a}	34.20 ± 1.38^{b}	4.57 ± 0.42^{b}	11.80 ± 0.61^{b}	4.73 ± 0.18^{b}	79.20±5.37	34.64±1.75	26.77±1.19
T1		1.80 ± 0.37^{a}	34.40 ± 1.86^{ab}	4.31 ± 0.51^{b}	11.54 ± 0.83^{b}	5.21 ± 0.20^{a}	82.81 ± 6.22^{a}	33.44 ± 1.21^{a}	27.60 ± 2.03^{a}
T2		1.80 ± 0.20^{a}	32.20 ± 1.36^{b}	4.40 ± 0.72^{b}	11.74 ± 0.92^{b}	4.96 ± 0.26^{b}	80.50 ± 11.10^{a}	36.69 ± 3.33^{a}	28.32 ± 2.64^{a}
T3		1.20 ± 0.20^{b}	37.80 ± 1.16^{ab}	5.14 ± 0.32^{a}	12.60 ± 0.38^{a}	4.87 ± 0.23^{b}	74.18 ± 2.33^{b}	33.33 ± 0.04^{b}	24.73 ± 0.79^{b}
T4		1.20 ± 0.20^{b}	38.80 ± 2.35^{a}	5.36 ± 0.60^{a}	12.94 ± 0.78^{a}	4.58 ± 0.32^{c}	74.40 ± 4.68^{b}	33.36 ± 0.03^{a}	24.82 ± 1.58^{b}
T1 x	BPR	1.33 ± 0.33^{c}	36.33 ± 1.67^{c}	4.80 ± 0.59^{b}	12.57 ± 0.13^{b}	5.02 ± 0.28^{b}	77.44 ± 6.87^{a}	34.70 ± 1.28^{a}	27.05 ± 3.47^{a}
T1x	IW	2.50 ± 0.50^{a}	31.50 ± 3.50^{d}	3.58 ± 0.85^{c}	10.00 ± 1.70^{d}	4.65 ± 0.46^{c}	90.87 ± 11.69^{a}	31.54 ± 1.89^{b}	28.44 ± 1.97^{a}
T2 x	BPR	2.00 ± 0.00^{a}	32.50 ± 2.50^{d}	3.69 ± 0.88^{c}	10.85 ± 0.85^{d}	5.12 ± 0.72^{a}	91.76 ± 15.00^{a}	33.38 ± 0.05^{a}	30.62 ± 4.96^{a}
T2 x	IW	1.67 ± 0.33^{b}	32.00 ± 2.00^{d}	4.87 ± 1.10^{b}	12.33 ± 1.45^{b}	4.22 ± 0.05^{c}	73.00 ± 16.30^{b}	38.89 ± 5.56^{a}	26.79 ± 3.46^{a}
T3 x	BPR	1.00 ± 0.00^{c}	40.50 ± 0.50^{a}	5.90 ± 0.03^{a}	13.50 ± 0.20^{a}	5.17 ± 0.57^{a}	68.64 ± 0.50^{b}	33.33 ± 0.08^{a}	22.88 ± 0.22^{b}
T3 x	IW	1.33 ± 0.33^{c}	36.00 ± 0.58^{c}	4.63 ± 0.13^{b}	12.00 ± 0.17^{c}	5.24 ± 0.14^{a}	77.87 ± 1.03^{a}	33.34 ± 0.05^{a}	25.96 ± 0.38^{a}
T4 x	BPR	1.00 ± 0.00^{c}	39.67 ± 2.73^{b}	5.60 ± 0.61^{a}	13.23 ± 0.91^{a}	5.07 ± 0.32^{b}	71.45 ± 2.65^{b}	33.36 ± 0.03^{a}	23.84 ± 0.89^{b}
T4 x	IW	1.50 ± 0.50^{b}	37.50 ± 5.50^{b}	5.01 ± 1.52^{ab}	12.50 ± 1.80^{b}	4.80 ± 0.57^{b}	78.82 ± 12.87^{a}	33.35 ± 0.09^{a}	26.30 ± 4.36^{a}
p- values (Breeds)		0.03	0.04	0.04	0.04	0.04	0.70	0.76	0.71
p- values (Treatments)		0.03	0.03	0.03	0.04	0.03	0.03	0.04	0.04
p- values (B×T)		0.04	0.04	0.04	0.02	0.03	0.03	0.04	0.04

^{a,ab,b,c,d}Means of the same column but with different superscripts are statistically significant (p<0.05); ±SEM= Standard Error Mean;WBV= Whole Blood Viscosity; BPR= Barred Plymouth Rock; IW= Isa White; T1= Diet with 0.25% salt; T2= Diet with 0.50 % salt; T3= Diet with 0.75 % salt; T4= Diet with 1.00 % salt; B = Breeds; T = Treatments.

Table III. Differential White Blood Counts of Barred Plymouth Rock and Isa White Cocks fed diets with varying levels of salt (Diferencial hemogramas de barrados Plymouth rocablanca y Isa blanco pollas alimentados con dietas con diferentes niveles de sal).

Treatments		Breed	Lym (%)	Het (%)	Mon (%)	Eos (%)	Bas (%)
		BPR	61.00±0.33	21.40±0.92	12.70±0.79	3.30±0.15^{a}	1.60±0.16^{b}
		IW	60.80±0.25	21.80±0.59	12.50±0.6	3.10±0.10^{b}	1.80±0.13^{a}
T1			60.80±0.58^{b}	22.20±1.20^{a}	12.20±0.58^{b}	3.20±0.20^{a}	1.60±0.24^{b}
T2			61.60±0.24^{a}	21.40±1.33^{a}	12.20±1.11^{b}	3.20±0.20^{a}	1.60±0.24^{b}
T3			60.60±0.40^{b}	22.00±0.84^{a}	12.40±1.03^{a}	3.40±0.24^{a}	1.60±0.24^{b}
T4			60.60±0.24^{b}	20.80±1.11^{b}	13.60±1.21^{a}	3.00±0.00^{b}	2.00±0.00^{a}
T1	x	BPR	60.67±0.88^{b}	22.00±2.00^{b}	12.67±0.88^{b}	3.33±0.33	1.33±0.33
T1	x	IW	61.00±1.00^{b}	22.50±1.50^{b}	11.50±0.50^{b}	3.00±0.00	2.00±0.00
T2	x	BPR	62.00±0.00^{a}	18.50±1.50^{d}	14.50±1.50^{a}	3.50±0.50	1.50±0.50
T2	x	IW	61.33±0.33^{b}	23.33±0.67^{a}	10.67±0.67^{c}	3.00±0.00	1.67±0.33
T3	x	BPR	61.00±1.00^{b}	23.50±0.50^{a}	10.50±1.50^{c}	3.50±0.50	1.50±0.50
T3	x	IW	60.33±0.33^{b}	21.00±1.00^{b}	13.67±0.88^{a}	3.33±0.33	1.67±0.33
T4	x	BPR	60.67±0.33^{b}	21.33±1.86^{b}	13.00±2.08^{a}	3.00±0.00	2.00±0.00
T4	x	IW	60.50±0.50^{b}	20.00±1.00^{c}	14.50±0.50^{a}	3.00±0.00	2.00±0.00
p- values (Breeds)			0.52	0.73	0.93	0.02	0.03
p- values (Treatments)			0.04	0.03	0.02	0.03	0.03
p- values (B × T)			0.03	0.01	0.01	0.80	0.70

[abcd]Means of the same column but with different superscripts are statistically significant ($p<0.05$); ±SEM= Standard Error Mean; BPR= Barred Plymouth Rock; IW= Isa White;Lym. = Lymphocyte; Het. = Heterophil; Mon.= Monocyte; Eos. = Eosinophil; Bas. = Basophil; T1= Diet with 0.25% salt; T2= Diet with 0.50 % salt; T3= Diet with 0.75 % salt; T4= Diet with 1.00 % salt; B = Breeds; T = Treatments.

decreased in the IW breed from 90.87±11.69fl in T1 to 78.82±12.87fl in T4. Also, the Hb increased in the BPR breed from 12.57±0.13g/dl in T1 to 13.23±0.91g/dl in T4 while it increased from 10.00±1.70g/dl in IW breed in T1 to12.50±1.80g/dl inT4. The breeds and the treatments however interacted to increase the WBV in both the BPR and the IW breeds.

The differential White Blood Counts are presented in **Table III**. The Table showed no difference (P>0.05) in lymphocyte, heterophil and monocyte components of the white blood counts in the Barred Plymouth Rock (BPR) and Isa White (IW) breeds. However differences in eosinophil (3.30±0.15% versus 3.10±0.10%) and basophil (1.60±0.16% versus 1.80±0.13%) content were observed respectively in the two breeds of cock. There were significant treatment effects in the differential leucocyte counts. The heterophil and eosinophil components of the WBC showed a decrease with increase in dietary NaCl levels while the monocytes and basophils showed a gradual increase with increasing levels of dietary NaCl levels. The highest monocyte (13.60±1.21%) and basophil (2.00±0.00%) levels were observed in T4. Similar trend was observed in the interactions between breeds and treatments among the various WBC components where for instance, the heterophils decreased from 22.00±2.00% in BPR breed in T1 to 21.33±1.86% in T4 while it decreased from 22.50±1.50% in IW breed (T1) to 20.00±1.00% (T4).

The values of osmotic fragility of cocks fed varying levels of dietary salt are presented in **Table IV.** There were no differences in the osmotic fragility of the red blood cells between the PBR and IW breeds. There were however significant treatment effects on osmotic fragility of the erythrocytes between 0.00 to 0.80% saline concentration. Birds on 0.50% dietary NaCl (T2) had a significantly higher osmotic fragility relative to other treatments across all saline concentrations. The general tendency was a significant increase in osmotic fragility relative to the control followed by a general decrease from T3 to T4 that made T1 and T4 to be statistically similar across all saline concentrations (0.00 to 0.90%). There were equally significant effects on the interactions between breeds and treatments on the erythrocytic osmotic fragility from 0.00 to 0.90% saline concentration. The diet with 0.50% NaCl (T2) was observed to provoke higher osmotic fragility in the two breeds of birds up to 0.80% saline concentration. There was however a reversal at 0.90% saline concentration where BPR on the T3 diet recorded the most osmotically fragile value (19.53±0.04%). In all cases, the BPR breed was observed to be the more osmotically fragile breed at all treatment and saline concentration levels.

DISCUSSION

The differences observed in the ESR, Hb, RBC, PCV and WBV between the two breeds of cock **(Table II)** could be linked directly to their different genetic linage. The IW and BPR breeds were derived from cross-breeding programs involving birds with diverse genetic make-up. The Isa for instance is a hybrid type of sex-linked chicken thought to originally be the result of a cross between Rhode Island Reds and Rhode Island Whites (http://www.backyardpoultrymag.com) in which the offspring were Isa White (cocks) and Isa

Table IV. Percentage erythrocytic Osmotic fragility of Barred Plymouth Rock and Isa White Cocks fed varying levels of dietary salt (La fragilidad osmotic eritrocitaria porcentaje de roca de Plymouth barrado y Isa blanco pollas alimentados con diferentes niveles de sal dietética).

		Saline Concentration									
Treatments	Breed	0.00	0.10	0.20	0.30	0.40	0.50	0.60	0.70	0.80	0.90
	BPR	51.89±3.99	48.61±3.87	44.57±3.59	40.40±3.56	34.49±3.59	29.84±3.38	24.60±2.47	19.54±2.08	12.93±1.72	0.00
	IW	52.41±4.47	46.91±2.92	42.76±2.21	38.46±1.96	33.07±1.93	28.51±1.95	24.28±1.72	19.20±1.86	13.32±1.55	0.00
T1		49.04±9.32[b]	42.60±6.83[c]	38.41±6.00[c]	33.60±5.74[b]	25.78±3.82[b]	20.66±3.28[c]	16.95±2.52[c]	12.73±2.20[c]	8.62±2.13[b]	0.00
T2		57.37±2.79[a]	54.22±3.07[a]	50.69±3.30[a]	46.51±3.52[a]	42.55±4.17[a]	37.55±3.80[a]	31.4±2.09[a]	25.92±1.71[a]	15.49±2.48[a]	0.00
T3		55.57±4.92[a]	50.07±2.96[b]	45.05±1.65[b]	41.33±1.61[ab]	35.59±1.99[ab]	31.67±2.16[ab]	26.44±1.46[ab]	20.94±1.82[ab]	15.18±2.02[a]	0.00
T4		46.62±4.81[b]	44.15±4.49[c]	40.51±2.97[c]	36.28±1.65[ab]	31.19±1.23[b]	26.81±0.58[bc]	22.97±0.5[bc]	17.89±1.09[bc]	13.19±1.39[ab]	0.00
T1 x	BPR	46.57±10.29[c]	42.61±9.64[c]	38.32±8.93[d]	34.77±9.23[c]	25.35±6.18[d]	20.96±5.74[d]	17.13±4.56[d]	12.97±4.01[d]	8.70±3.81[d]	0.00
T1 x	IW	52.75±22.97[b]	42.57±13.68[c]	38.54±10.98[d]	31.85±8.29[d]	26.43±5.54[cd]	20.21±2.87[d]	16.67±1.12[d]	12.37±0.37[d]	8.49±1.28[d]	0.00
T2 x	BPR	62.86±5.07[a]	59.75±6.52[a]	55.97±7.68[a]	51.39±9.18[a]	48.01±11.13[a]	41.53±10.73[a]	32.90±6.29[a]	25.73±4.44[a]	11.14±3.31[d]	0.00
T2 x	IW	53.71±0.78[b]	50.53±0.36[b]	47.16±1.05[b]	43.26±0.20[a]	38.90±0.20[a]	34.89±0.99[a]	30.39±0.38[a]	26.04±1.76[a]	18.39±2.53[a]	0.00
T3 x	BPR	55.19±1.04[b]	51.52±0.80[b]	47.95±1.51[b]	44.02±2.91[a]	38.10±3.05[a]	34.70±3.56[a]	28.86±1.10[a]	24.11±0.80[a]	19.53±0.04[a]	0.00
T3 x	IW	55.82±8.97[b]	49.10±5.27[b]	43.11±1.90[c]	39.54±1.35[b]	33.92±2.56[b]	29.65±2.50[b]	24.83±1.85[b]	18.83±2.29[b]	12.29±1.74[b]	0.00
T4 x	BPR	47.69±7.60[b]	45.24±7.03[c]	40.95±4.43[d]	36.28±2.18[c]	32.21±0.84[b]	27.68±0.41[b]	23.68±0.20[b]	18.94±0.91[b]	13.94±1.14[b]	0.00
T4 x	IW	45.01±7.32[c]	42.52±7.01[c]	39.84±5.33[d]	36.27±3.61[c]	29.67±3.04[c]	25.51±0.28[c]	21.9±0.71[c]	16.31±2.29[c]	12.08±3.67[c]	0.00
p-values (B)		0.86	0.50	0.41	0.35	0.28	0.21	0.30	0.30	0.79	0.00
p-values (T)		0.04	0.03	0.04	0.03	0.02	0.02	0.04	0.03	0.04	0.00
B×T		0.03	0.03	0.03	0.04	0.02	0.02	0.03	0.03	0.02	0.00

[a,ab,b,bc,c,cd,d]Means of the same column but with different superscripts are statistically significant ($p<0.05$); ±SEM= Standard Error Mean. BPR= Barred Plymouth Rock; IW= Isa White; T1= Diet with 0.25% salt; T2= Diet with 0.50 % salt;T3= Diet with 0.75 % salt; T4= Diet with 1.00 % salt; B = Breeds; T = Treatments.

Brown (hens). The Barred Plymouth Rock was also a breed claimed to have been developed from a cross-breeding program involving several breeds like Dominiques, Java Black, Cochins, Malays and Dorkings (http://www.Plymouth rock.web.com/standard.htm). Such breed differences in the commercial lines of egg-type birds have also been observed in terms of wetness of litter (Choct et al. 1999) and sodium chloride tolerance (Chen & Balnave, 2001). The WBV decreased across the treatments relative to the concentration of NaCl in the diets. This blood viscosity lowering ability of dietary salt had been reported by Aro and Akinleminu (2015) in layers and by Windberger et al. (2003) in cattle, sheep, rabbit and mouse. The higher ESR but lower WBV in the IW cocks than in the BPR cocks implied that the IW cocks are likely to adapt better in the face of blood circulatory challenge with a lower endothelial shear stress on the blood capillaries than the BPR breed (Pfafferotti et al. 1999). The observed increase in RBC due to the treatment effect was supported by the report of Mcleod (2010) that increase in dietary consumption NaCl could increase the RBC.

Though there were no breed differences in the lymphocyte, heterophil and monocyte components of the WBC, the eosinophil and basophil components differed between the two breeds **(Table III)**. Choct et al. (1999) observed such differences in the commercial lines of Isa Brown, Hisex and Hy-line breeds and concluded that the disparity in the wetness of litter in these breeds was genetic in origin. The observed decrease in heterophil (neutrophil) and eosinophil components of the WBC with increase in dietary salt could be ascribed to their common haematopoetic linage. Since both are from the myeloblast progenitor (Jain 1993), both could be expected to react similarly to direct dietary NaCl assault. The improvement in the monocyte and basophil content of WBC with dietary salt level may highlight the claim that NaCl is required not only as an appetiser but also as an immunity booster in the body. This claim is supported by the report of Moltu et al. (2013) that enhanced feeding could cause electrolyte disturbances which may affect monocytes and neurophils that are important in the defence against bacterial infection. However, since basophils are a rarity in the blood (Jain 1993), values as high as 2.00% in the white blood cells as observed under the current study could therefore indicate a case of basophilia at 1.00% of dietary salt in the feed.

The values of osmotic fragility presented in **Table IV** showed a decrease in the percentage of red blood cells haemolysed per saline concentration from 0.90 % to 0.00 %. This is in consonance with the report of Aro et al. (2013) on broiler's red blood cells. This experiment further confirmed the report of Oyewale (1994) and Aldrich et al. (2006) that complete haemolysis cannot be ensured even at the least saline concentration as far as the osmotic fragility of the avian and reptilian red blood cell is concerned. This is caused by the nucleated red blood cells of these species that confer better resistance to fragility in hypotonic medium than in their mammalian counterparts. The osmotic fragility of the red blood cells of the birds showed that those on the control diet had lower fragility in comparison with those on the high salt diets. But again, this disparity is not dependent on the salt content of the feed as T2 with lower salt content had a higher fragility than T4 with 1.00% salt content. It could also be observed from the Table that the Isa White breed seemed to be more osmotically stable than the Barred Plymouth Rock breed when their erythrocytic fragilities are called to question.

CONCLUSION

The study showed that breed differences exist between the Barred Plymouth Rock (BPR) and Isa White (IW) in erythrocyte sedimentation rate, packed cell volume, haemoglobin, mean corpuscular volume and whole blood viscosity. Also these haematological indices were both positively and negatively influenced by varying levels of NaCl in the diets. Feeding high salt diets to breeding BPR and IW cocks had no deleterious effects on their leucogram as far as the lymphocytes, heterophils, monocytes and eosinophils are concerned but a condition of basophilia could be observed when the salt content in the feed of these birds is increased to 1.00%. The high salt diets lowered the osmotic stability of the red blood cells. It could also be concluded that the IW is the more osmotically resistant breed in terms of erythrocytic osmotic fragility.

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