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Deprivation of Drinking Water for up to 48 Hours Does Not Affect the Osmotic Fragility of Erythrocytes from Captive Helmeted Guinea Fowl (*Numida meleagris*)

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Abstract: Poor management practices may result in poultry having inadequate drinking water for prolonged periods and consequently adverse haematological effects. The effects of water deprivation for up to 48 hours on osmotic fragility of erythrocytes, plasma osmolarity and erythrocyte indices was investigated in eighteen, female Guinea fowl. Six birds had drinking water *ad libitum* and the other 12 were deprived of drinking water for up to 48 hours. Erythrocyte osmotic fragility was determined in serially diluted phosphate buffered saline. Deprivation of drinking water significantly ($p < 0.05$, ANOVA) decreased body mass ($-7.9 \pm 2.4\%$ and $-12.8 \pm 3.2\%$, after 24 and 48 hours, respectively) but did not ($p > 0.05$) affect erythrocyte osmotic fragility. Water deprivation significantly ($p < 0.05$) increased plasma osmolarity and PCV but decreased the MCV. However, there was no significant difference ($p > 0.05$) in the osmolarities, PCVs and MCVs of the birds deprived of drinking water for 24 hours compared to those deprived for 48 hours.

Key words: Guinea fowl, water deprivation, erythrocyte osmotic fragility

Introduction

The ability of an animal to respond to osmotic challenges associated with cyclic dehydration and rehydration is reflected in the mean corpuscular fragility of its erythrocytes (Buffenstein *et al.*, 2001). The osmotic fragility of erythrocytes is a measure of their overall response to osmotic pressure and is influenced by several intrinsic and extrinsic factors. The osmotic resistance of erythrocytes of some adult animals has been reported to occur in the following decreasing order of resistance; camel, chicken, dog, pig, rabbit, guinea pig, mouse, rat, hamster, horse, donkey, ox, cat, sheep and goat (Perk, 1964). Amongst birds, there are species differences in the osmotic fragility of erythrocytes in response to water deprivation. Depriving ducks of water for 24 hours resulted in an increased erythrocyte osmotic fragility (Baloyi *et al.*, 2006). Yagil *et al.*, 1976, showed that keeping chickens at 35°C without drinking water resulted in haemoconcentration and increased erythrocyte osmotic fragility. On the other hand, the erythrocytes of turkey poults deprived of water for 3 days were resistant to haemolysis (Augustine and Witlock, 1983). Osmotic fragility of avian erythrocytes is also affected by hormones and gender (March *et al.*, 1966). The presence of nucleated red blood cells in birds makes regulation of cellular osmolarity and transport processes a more intricate process than in mammals. The ion transporters on nucleated avian red blood cells are affected by stress hormones, unlike mammalian red

blood cells (Lytle, 1998). Injecting chickens with diethylstilbestrol was observed to increase the proportion of oleic and linoleic acid and also increased resistance of erythrocytes to osmotic haemolysis (March *et al.*, 1966). Conversely, the effect of testosterone was opposite to that of oestrogen (March *et al.*, 1966). Hypothyroidism in birds increases osmotic fragility of the erythrocytes (Dariyerli *et al.*, 2004). Other factors that affect the resistance of avian erythrocytes to osmotic haemolysis include disease ((Perk, 1964; Augustine and Witlock, 1983) and metabolic inhibitors like cycloheximide, puromycin, actinomycin-D and EDTA (Moody *et al.*, 1977).

Guinea fowl farming is popular amongst smallholder farmers in Africa (Nwagu and Alawa, 1995). Guinea fowl have an attractive plumage, game-type meat flavour and high meat to bone ratio (Embury, 2001). They also have a greater capacity to scavenge for insects and grains, a higher ability to protect against predators and greater resistance to common poultry parasites and diseases like Newcastle disease and fowl pox than chickens (Micro livestock, 1991). Despite the importance of Guinea fowl in poultry farming, research is generally focused on the chicken and findings extrapolated to guinea fowl. There is a dearth of literature on how water deprivation affects the fragility of guinea fowl erythrocytes. The aim of this study was to determine the effects of depriving Guinea fowls of drinking water for up to 48 hours on red blood cell osmotic fragility, haematocrit and plasma osmolarity.

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Table 1: Ingredient composition (g.kg⁻¹) and calculated nutrient composition of the diet fed to the guinea fowls

Ingredient	g.kg ⁻¹
DL-Methionine	0.5
Limestone	74.6
Maize	622.5
Mono-Calcium Phosphate	15.8
Soya bean Meal	225.4
Salt	2.5
Sunflower Cake	58.7
Calculated Analysis (g.kg ⁻¹ except where indicated otherwise)	
Dry Matter	883.3
Crude Protein	161.5
Crude Fibre	46.6
Ether Extract	36.5
Metabolizable Energy (Kcal.Kg ⁻¹)	2700
Lysine	8.0
Methionine	3.4
Methionine+cystine	6.1
Calcium	31.9
Phosphate (available)	7.0

Materials and Methods

This study was approved by the University of Zimbabwe, Faculty of Veterinary Science research committee, which ensured that ethical procedures and minimum animal care standards were adhered to during the study which was undertaken in 2005.

Guinea fowl, housing and feeding: Eighteen, female, six month old Guinea fowl (*Numida meleagris*), that had been bred and hatched at Henderson research station (a Zimbabwean government research institute, located about 40 kilometres East of the capital city Harare), were used in this study. The guinea fowl were fed ad libitum on a standard diet formulated to supply a balance of nutrients appropriate to the age of the birds (Table 1). After a two-week acclimatization period (to the diet and the housing conditions) the guinea fowl were randomly divided into three groups; a control group which received drinking water ad libitum, a group that was deprived of drinking water for 24 hours and the third group which was deprived of drinking water for 48 hours. The three groups were further split into 2 groups of three birds each and were all kept in cages measuring 1.5m×0.8m×0.5m. The temperature and relative humidity in the cages housing the birds was maintained at 28°C and less than 30% respectively during the study period. The mass of the birds was measured immediately before and after water deprivation. The birds had *ad lib* access to feed for the duration of the experiment.

Blood collection: Blood was collected (8 mL) before water deprivation (0 hours) after 24 hours in the second group and after 48 hours of water deprivation in the third group. It was collected by venipuncture from the Jugular

vein using heparinized 10 mL syringes and 20G needles. The blood was immediately placed in sterile Li Heparin tubes which were stored at room temperature and tests were performed within 20 minutes of collection.

Determination of osmotic fragility: Osmotic fragility of the erythrocytes in vitro was determined by measuring the release of haemoglobin from blood added to tubes containing serially diluted phosphate buffered saline (Oyewale, 1991). In summary, 20 microlitres of blood was added to tubes containing 5 mL of phosphate buffered saline (pH 7.4) of serial concentrations ranging from 0-0.85% Phosphate Buffered Saline (PBS). The mixtures were allowed to stand for 60 minutes at room temperature (24°C) and then centrifuged (Heraeus Omnifuge, Germany) at 1580g for 5 minutes. The supernatant was decanted and its haemoglobin was determined spectrophotometrically (LKB ultrospec II, LKB Biochrom Ltd, England) at 540nm using distilled water as a blank. The percentage of haemolysis in each concentration of PBS was calculated assuming 100% haemolysis in the concentration with the highest absorbance. All measurements were replicated twice.

Determination of plasma osmolarity and erythrocyte indices: A cryoscopic osmometer (Osmomat 030, Gonotec GmbH, Berlin, Germany) was used to determine the osmolarity of the plasma samples. Packed cell volume was measured using microhaematocrit capillary tubes and centrifuged (Heraeus Omnifuge, Germany) at 1580g for 5 minutes. The Mean Corpuscular Volume (MCV) was determined using a coulter counter-T890[®] (Coulter Electronics Ltd, England).

For each sample of blood a smear was made on a glass slide, air dried and then stained with modified Giemsa stain using an automatic staining machine (Hematek 1000[®], AMES, Miles Laboratory, USA). The smears were observed with a microscope and evaluated for abnormalities of red blood cells, leucocytes and thrombocytes and, for the presence of blood parasites.

Statistical analysis: All data are expressed as mean±Standard Deviation (SD). One way analysis of variance followed where differences were observed by Tukey-Kramer multiple comparisons test was used. The level of significance was reported at p<0.05.

Results

Body mass changes: Depriving the guinea fowls of water for 24 and 48 hours resulted in a significant decrease (ANOVA, p<0.01) in body weights of the birds (Table 2).

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Table 2: Effect of water deprivation for 0, 24 and 48 hours on body mass, PCV, MCV and Plasma osmolality (n = 6 for each group)

Parameter	Control (0Hrs water deprivation)	24 Hrs water deprivation	48 Hrs water deprivation
Change in body mass from initial (%)	0.0±0.0 ^a	-7.9±2.4 ^b	-12.8±3.2 ^b
PCV (%)	33.3±1.8 ^a	35.8±2.6 ^{ab}	37.3±2.4 ^b
MCV (fl)	177.6±3.6 ^a	170.2±0.8 ^b	170.4±0.3 ^b
Osmolarity (mOsm.L ⁻¹)	336.3±12.1 ^a	353.5±7.6 ^b	364.3±7.2 ^b

^{a,b}Data in same row with different superscript indicates significant difference (p<0.05)

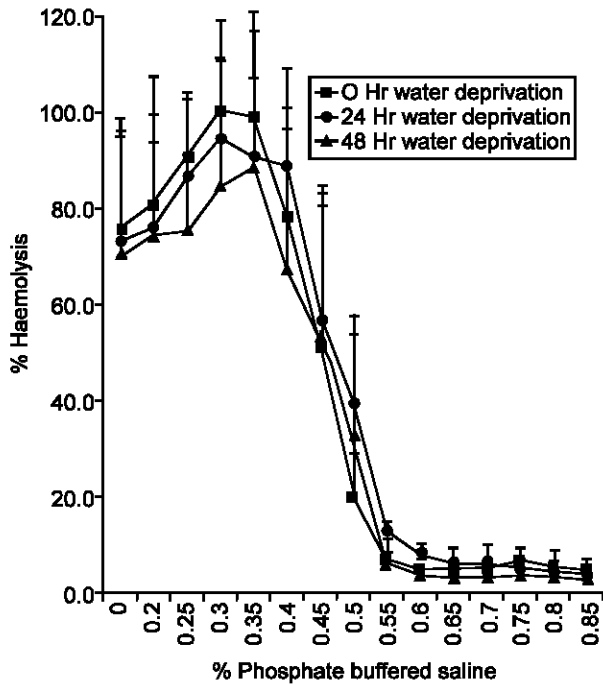


Fig. 1: The effect of water deprivation on osmotic fragility of red blood cells from Guinea fowl

Erythrocyte fragility: Depriving guinea fowls of water for up to 48 hours did not have a significant ($p>0.05$) effect on erythrocyte osmotic fragility (Fig. 1). The fragiligrams for blood collected after 0, 24 and 48 hours of water deprivation showed that initial haemolysis (>4%) occurred within 0.55% to 0.60% PBS. The mean corpuscular fragility (50% haemolysis (Suess *et al.*, 1984)) of red blood cells occurred between 0.45% and 0.50% and maximum haemolysis occurred between 0.3% and 0.4% saline for the three groups of guinea fowl (Fig. 1).

Plasma osmolality and erythrocyte indices: Water deprivation significantly ($p<0.05$) increased the osmolality of the plasma (Table 2). However, there was no significant difference ($p>0.05$) in osmolality of the birds deprived of drinking water for 24 hours and those deprived for 48 hours. Depriving the guinea fowl of water significantly ($p<0.05$) increased the PCV. There was however, no significant difference ($p>0.05$) between the birds deprived of water. Similarly, there were significant

differences ($p<0.05$) in MCV of water deprived birds compared to control, but with no significant difference ($p>0.05$) between the birds deprived of water for 24h compared to 48h.

Blood smears: No abnormalities in erythrocyte morphology or parasites were observed.

Discussion

This study showed that depriving Guinea fowl of water for up to 48 hours did not affect the osmotic fragility of their erythrocytes which is in contrast to the findings in other birds such as chickens (Yagil *et al.*, 1976) and Pekin ducks (Baloyi *et al.*, 2006), where water deprivation increased osmotic fragility. The observed maximum fragility of the guinea fowl erythrocytes occurring between 0.3 and 0.4% PBS is in agreement with earlier findings for birds (Lewis and Ferguson, 1966). However, studies on the osmotic fragility of erythrocytes of pea fowls, pigeons and ducks (Oyewale, 1993; Baloyi *et al.*, 2006) showed initial haemolysis at room temperature in 0.5% saline, whereas our study on guinea fowl shows this value at between 0.55-0.60% saline. By depriving the birds of drinking water, our study was premised on *in vivo* manipulation of the birds. Previous studies on the fragility of Guinea fowl erythrocytes which investigated the effects of *in vitro* manipulations e.g. change in pH and temperature of the buffer solutions, showed an increase in fragility at higher temperatures and pH and significant differences between chickens, Guinea fowl and ducks (Oyewale *et al.*, 1991; Oyewale, 1993).

The PCV values that we obtained for the control group are in agreement with earlier findings by Uko and Ataja (1996) who reported values ranging between 31.4±0.7% to 34.3±0.6% in Guinea fowl with free access to water. Oyewale and Ogweugbu (1986) reported higher values of 37.93±1.75% for female guinea fowl with the male fowl in their study having higher PCVs than the females. PCV is affected by several factors e.g. hormones, altitude, hydration status, acceleration, age, sex and hypoxia (Sturkie and Griminger, 1986).

The MCV values we recorded were also lower than those reported by Oyewale and Ogweugbu (1986) of 197±11.05fL. According to Uko and Ataja (1996) who reported a similar trend in MCV, the smaller cell volume of the guinea fowl red blood cell might explain the difference seen between the rate of haemolysis of the red blood cells of chickens and the guinea fowl.

Although increased fragility is reported to be positively correlated with decreased cell volume (Jain, 1986), this study did not show any significant difference in osmotic fragility despite the differences in MCV between the control and the groups deprived of water for 24 and 48 hours.

The plasma osmolarity values are also comparable with those reported for chickens given water ad libitum (Yagil *et al.*, 1976). Although the increase in PCV (haemoconcentration) and osmolarity observed after 24 and 48 hours of water deprivation is a common finding in both mammals and most species of birds deprived of water, it is interesting to note that the haematocrit and plasma osmolarity in ostriches has been reported to be so well regulated that water deprivation for up to eleven days resulted in insignificant haemoconcentration (Brown and Jones, 1996). Proximate analysis of the feed showed a dry matter content of 88.3% (Table 1) implying about 12% water content, the increase in plasma osmolarity and loss of body mass that we observed supports the inference that the feed was unable to provide enough water to meet requirements for a state of euhydration of the captive guinea fowl deprived of water. Lab experiments have shown that for most granivorous birds fed only dry seeds (water content about 10%), total water deprivation resulted in either an initial weight loss with subsequent survival for months or continual weight loss and death after a period during which the food intake declined (Skadhauge, 1981).

The haemoconcentration as a result of water deprivation could also be responsible for the decrease in MCV that we observed.

The resistance of red blood cells to osmotic haemolysis may be affected by disease and blood parasites (Perk, 1964). In this study no blood parasites were observed in all the samples examined.

Further studies are recommended to investigate the effect of factors such as age, sex, diet, vaccination, transportation and seasonal variation on osmotic fragility of guinea fowl red blood cells.

We have shown that depriving Guinea fowl of drinking water for up to 48 hours did not affect the osmotic fragility of red blood cells unlike findings reported in chickens and ducks, but it affected PCV, MCV and osmolarity. It is thus important to interpret haematological parameters cautiously in Guinea fowl and not just extrapolate data from chickens or ducks.

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