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RESEARCH

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Physiological responses of Lizard, Toad and Pigeon during rainy season in tropical savanna climate

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Abstract

Background This study investigated the adaptation mechanisms of Pigeons, Toads and Lizards during the rainy season in tropical savanna climates. Male Agama Lizards (*Agama agama*), Afep Pigeons (*Columba unicincta*) and Toads (*Sclerophrys perreti*) were captured at a location in Ibadan, Oyo state. Five (5) Lizards marked: L1 to L5, Afep Pigeons marked: B1 to B5 and Toads marked: T1 to T5. Each of the animals was manually constrained within six to eight hours of capture, and a 1.0-ml syringe was used to obtain blood from the heart or coccygeal vein. Blood samples were collected in an EDTA and plain bottle, respectively. Plasma and sera samples were separated by centrifugation at − 4 °C using a cold centrifuge and then analysed for creatinine, urea, glucose, Mg^+, k^+, $Cl^−$, Na^+, albumin, TP, ALP, ALT, AST, GGT, MDA, SOD, CAT, GSH and haematological parameters. Cervical dislocation was performed on the animals before organs such as kidneys and liver were collected from each of the animals. Each organ collected from each animal was placed in separate plain tubes (filled with phosphate buffer).

Result The Pigeon had higher body temperature, urea, glucose, ALP, PCV, haemoglobin concentration, neutrophil and triglyceride compared to the Lizard and Toad, while the Toad had higher Na^+, Mg^+ and $Cl^−$, basophil and monocytes compared to Pigeon and Lizard and the Lizard has higher creatinine, lymphocyte and cholesterol compared to the Pigeon and Toad. On the liver oxidative stress markers, the Pigeon has higher superoxide dismutase and reduced glutathione compared to the Lizard and Toad, while the Toad has higher MDA and catalase compared to the Pigeon and Lizard. On the kidney oxidative stress markers, the Pigeon has higher MDA compared to the Lizard and Toad, while the Toad has higher catalase than the Pigeon and Lizard.

Conclusions From this study, the elevated level of lymphocytes in Lizard and eosinophil, basophil and monocytes in Toad suggests that Lizards and Toad are more vulnerable to inflammation. The high value of cholesterol in Lizard and triglyceride in Pigeon as observed in this study may relate to the degree of stress. Also, the activation of antioxidant systems under comparative study is a part of the survival strategy of animals like amphibians, reptiles and aves when facing environmental problems.

Keywords Oxidative stress markers, Creatinine, Cholesterol

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Background

Adaptation is simply the natural changes in physiology, structure and behaviour in an organism to fit an environment (Corrigan, 2017). Evolutionary adaptions such as colouration, body shape, jaw or beak shape and forelimb structure support the survival of an organism in a particular environment (Klir & Heath, 1994; Sánchez-Guzmán et al., 2004). The understanding of a particular reptile, amphibian and aves species is based on the biochemical parameters majorly. The shortfall in the availability of already established data hinders the proper understanding of the biochemical adaptation of different species. Various studies show the effect of season on biochemical blood parameters (Ribeiro et al., 2016; Sejian, 2011). In addition, the combination of relative humidity (RH) and high ambient temperature comprises the animal's ability to lose heat to the environment and may explain the cause of heat stress (Aziz et al., 2016). Animals show many biochemical and endocrine adjustments to reduce the stress caused by different environmental factors. This gives rise to acclimation which is achieved through acclamatory homeostasis if heat stress is prolonged (Horowitz, 2002). Adaptation is one of the main agenda of comparative physiology (Rezende & Diniz-Filho, 2012). The field of comparative physiology deals with the research of physiological ethics via the investigation of functional characteristics of diversified organisms. Part of the importance of this subdivision in physiology is to derive what ecological pressure had resulted in notable physiological adaptation possessing a selective advantage and how selective pressures have created differences and similarities observed among different species (Gilmour et al., 2005).

The *Agama agama* belongs to the Agamidae family of Lizards with over 300 species of Iguana Lizards (Wagner, 2009). They are native to Africa, Asia, Australia and a few in Southern Europe. The Afep Pigeon (*Columba unicincta*) also referred to as African Wood Pigeon is a member of the Columbidae family (Heine, 1858, 1860). They live in the areas of the African Tropical rainforest. The *Sclerophrys perreti* is a Toad species in the Bufonidae family endemic to South Western Nigeria (Onadeko et al., 2014).

Ibadan is a prominent city in western Nigeria located in the tropical savanna climate zone. The tropical grasslands are habitat to many animal species such as reptile species, lions, antelopes, tigers, aves and amphibians. This city is situated in the centre of the derived savannah and the tropical forest zones. Tropical savanna climates lie within the tropical latitudes, hence they are relatively hot. Koppen climate classified that it has a tropical wet and dry climate with a mean total rainfall of 1420.06 mm, mean peak temperature of 26.46 °C and mean least temperature of 21.42 °C (Saunders et al., 2015).

The measurement of physiological, haematological and biochemical adaptation parameters is essential in the monitoring and evaluation of the health of domestic and wild populations (Arguedas et al., 2018; Lewbart et al., 2015). Identifying the potential impacts of diseases, pollutants, injury and changing environmental conditions is not possible without a good knowledge of variations in haematological and biochemical parameters across species (Seebacher, 2005). The unavailability of biochemical parameters for the *Agama agama*, *Sclerophrys perreti* and the Afep Pigeon (*Columba unicincta*) is a limitation to the understanding of the physiological adaptation of these species to the tropical savannah climate.

Reptiles, birds and Toads are part of the food web in most ecosystems; they disperse seeds and act as pollinators. They can also be important in controlling agricultural pests, which gives the social and ecological relation to man (Ajayi & Olaleye, 2020). An understanding of the adaptation of these species will be of immense benefit to man socially and economically.

A thorough search of literature yielded few studies focussing on the biochemical parameters of these species about environment. The lineages of animals may be influenced by climate. Animals are also sensitive to climate change and this justifies the current trend that could threaten these species. Therefore, we ask the question of how biochemical parameters have responded to the tropical savanna climate in which these animals are found and carry out a comparative study on the three species.

This study will help to understand diversity in adaptation and probe for contrasts in the adaptation mechanism evolved by each species to survive comfortably in their habitats.

Methods

Ethical approval

Approval of this research project was granted by the Research Ethics Review Committee, Faculty of Basic Sciences, Lead City University, Ibadan, Nigeria.

Experimental animals and design

Male Agama Lizards (*Agama agama*), Afep Pigeons (*Columba unicincta*) and Toads (*Sclerophrys perreti*) were captured at a location in Ibadan, Oyo state (temperature: 22°–25°; humidity: 94–100%) on 13th of September 2022.

Five Lizards were captured and marked: $L1$ = lizard one, $L2$ = lizard two, $L3$ = lizard three, $L4$ = lizard four, $L5$ = lizard five.

Five Afep Pigeons were captured and marked: *B1* = bird one, *B*2 = bird two, *B*3 = bird three, *B4* = bird four, and *B*5 = bird five.

Five Toads were captured and marked: *T*1 = toad one, *T*2 = toad two, *T*3 = toad three, *T*4 = toad four, *T*5 = toad five.

Each was placed inside a cloth bag and transported to Lead City University Physiological Laboratory for sample collection.

A digital weighing balance was used to determine the body weight of the animals. An infrared digital thermometer was used to determine the temperature of the animals. The respiratory rates of the animals were determined manually by counting the movements of the abdominal cavity of the animals per minute.

Haematology and biochemical parameters

Each of the animals was manually constrained within six to eight hours of capture, and a 1.0-ml syringe was used to obtain blood from the heart or coccygeal vein. The blood samples were immediately divided into two bottles: (1) blood samples collected in an EDTA tube and (2) blood samples collected in a plain bottle. Cervical dislocation was performed on the animals before organs such as kidneys and liver were all collected from each of the animals. Part of each organ collected from each animal was placed in a separate plain tube (filled with phosphate buffer) to be homogenized for biochemical analysis. Another section of the liver and kidney was fixed in 10% neutral-buffered formalin (volume/volume). The blood samples for each animal collected in plain bottles were allowed to clot and then centrifuged at − 4 °C using a cold centrifuge for 5 min at 4000 rpm to collect the serum which was analysed for biochemical variables such as serum creatinine, urea, glucose, Mg^{+}, k^{+}, Cl^{-} and Na^{+} levels using standard laboratory methods (Ajayi et al., 2019). Serum ALP, ALT, AST, GGT, TP and albumin used previously described methods (Nwaji et al., 2022). The blood in the EDTA tube was assayed for haematological parameters using standard laboratory techniques.

Oxidative stress assay

Oxidative stress markers (MDA, SOD, CAT GSH and GSSG) were assayed by established methods (Ajayi et al., 2019) from the homogenates of the liver and kidney of the three animals.

The other sections of the organ fixed were thereafter embedded in paraffin wax. A resultant section thick in about 5–6 µm was stained with haematoxylin and eosin for histological assessment according to the standard method (Ogihara & Okabe, 1993).

Table 1 Physiological Variables of Lizard, Pigeon and Toad

Physiological variables	Lizard	Toad	Pigeon
Body temperature	24.62 ± 0.12	22.64 ± 1.86	41.56 ± 0.43$^{\$\ \#}$
Respiratory rate	45.80 ± 6.45	45.20 ± 4.72	51.60 ± 4.87

$ Significantly different compared to the Lizard

\# Significantly different compared to Toad

Table 2 Showing renal function tests, glucose and electrolytes levels in the serum of Lizard, the *Slerophrys perreti* and the *Columba unicinta*

Biochemical assay	Lizard	Toad	Pigeon
Creatinine (mg/dl)	351.20 ± 73.66$^{£,\ \#}$	8.24 ± 1.88	2.56 ± 0.30
Urea (mg/dl)	1.42 ± 0.03	99.20 ± 4.53	101.40 ± 23.16$^{\$,\ \#}$
Glucose (mg/dl)	97.74 ± 12.86	108.00 ± 11.52	214 ± 18.81$^{\$,\ \#}$
Magnesium (mg/dl)	1.98 ± 0.06	2.26 ± 0.05$^{\$,\ £}$	1.70 ± 0.22
Chloride (mEq/l)	99.01 ± 7.70	244.20 ± 30.34$^{\$,\ £}$	129.00 ± 8.57
Potassium (mEq/l)	7.72 ± 4.12	13.12 ± 4.27	11.52 ± 2.70
Sodium (mmol/l)	128.00 ± 15.16	302.80 ± 56.02$^{\$,\ £}$	269.40 ± 28.34

$ Significantly different compared to the Lizard

£ Significantly different compared to Pigeons

\# Significantly different compared to Toad

Data analyses

Data from this study were expressed as Mean ± SEM (standard error of the mean). The Graph Pad Prism software for Windows (version 7) was used for statistical analysis. One-way analysis of variance (ANOVA) was used to ascertain the differences between the animal groups and statistical significance was set at $P < 0.05$.

Results

Comparative analyses of physiological variables of Lizard, Lizard and Toad

There was no significant difference in the body temperature between Lizard and Toad. However, Pigeon shows significantly higher body temperatures when compared with Lizards and Toads. There is no significant difference in the respiratory rate across the groups. The results of body temperature and respiratory rate are presented in Table 1

Comparative analyses of the renal function indices, glucose and electrolytes levels in serum of Lizard, Slerophrys perreti and Pigeon

Table 2 shows that the creatinine level in Agama Agama was significantly higher than that in the Toad and Pigeon, respectively. However, no significant difference was seen in Toad and Pigeon creatinine levels. There was a significantly higher level of urea in the sample of Pigeon compared with the level observed in the samples of Lizard and Toad, respectively. There was no significant difference in the blood glucose level measured in Lizard and Toad. However, there was a significantly higher level of blood glucose observed in Pigeon compared with Toads and Lizards, respectively. There was a significantly higher level of magnesium ion observed in Toads, compared with that of Lizard and Pigeon. In the same vein, chloride ion was significantly higher in Toad compared with the levels in Pigeon and Lizard. However, there was no significant difference in the potassium ion level across the group. The sodium ion level was significantly higher in *Slerophrys perreti* compared with the level in Columba *unicinta* and Lizard.

Comparative analyses of the liver function indices of Lizard, Slerophrys perreti and *Columba* unicinta

In Table 3, the result showed that there was no significant difference in the level of AST and ALT across the groups. There was no significant difference in the level of ALP between the Lizard and the Toad. However, a significant increase was observed in the ALP of Pigeon compared with Toad and Lizards. Total protein level showed no significant differences across the group. There were also no significant differences in the level of albumin and GGT across the groups.

Table 3 Results of liver function indices of Lizard, *Slerophrys perreti* and *Columba unicinta*

Biochemical assay	*Agama agama*	*Slerophrys perreti*	*Columba unicinta*
AST (u/l)	23.04 ± 8.78	42.00 ± 16.97	17.40 ± 4.56
ALT (u/l)	13.96 ± 4.56	51.40 ± 17.85	25.00 ± 4.12
ALP	22.92 ± 1.92	19.60 ± 2.20	55.00 ± 2.42$^{\$, \#}$
Total protein (g/dl)	6.64 ± 1.10	9.54 ± 0.47	8.38 ± 0.79
Albumin (g/dl)	2.88 ± 0.22	3.72 ± 0.46	3.34 ± 0.27
GGT (u/l)	15.54 ± 5.51	16.92 ± 6.73	9.52 ± 1.70

[$] Significantly different compared to the Lizard

[#] Significantly different compared to Toad

Comparative analyses of oxidative stress markers on the liver of Lizard, Slerophrys perreti and *Columba* unicinta

There was a significant increase in the levels of malondialdehyde in *Slerophrys perreti*'s liver, compared with the liver of Lizard and *Columba unicinta*. *Slerophrys perreti*'s liver showed a higher level of catalase compared with the livers of Lizard and Columba unicinta, respectively. Superoxide dismutase was significantly higher in *Columba unicinta*'s liver compared with the levels observed in *Slerophrys perreti* and Lizard. Reduced glutathione was higher in *Columba unicinta*'s liver compared with the liver of *Slerophrys perreti* and Lizard, respectively. The level of Glutathione disulphide was higher in the liver of *Slerophrys perreti* and *Columba unicinta* compared with that of the Lizard. The results of the liver oxidative stress markers are displayed in Table 4**.**

Comparative analyses of oxidative stress markers in kidney of Lizard, Slerophrys perreti and *Columba* unicinta

Table 5 shows that malondialdehyde was significantly higher in *Columba unicinta*, compared with the levels observed in Lizard and *Slerophrys perreti*. The level of catalase in *Slerophrys perreti*'s kidney was significantly higher, compared with the level observed in the kidneys

Table 4 Oxidative stress markers on liver of Lizard, *Slerophrys perreti* and *Columba unicinta*

Oxidative stress markers	Lizard	*Slerophrys perreti*	*Columba unicinta*
Malondialdehyde (mM)	4.84 ± 0.54	8.38 ± 0.64$^{\$, £}$	5.30 ± 0.88
Catalase (u/mg)	2.06 ± 0.40	58.56 ± 8.93$^{\$, £}$	6.26 ± 1.17
Superoxide dismutase	0.11 ± 0.03	3.60 ± 0.97	11.60 ± 2.71$^{\$, \#}$
Reduced glutathione	0.19 ± 0.01	0.17 ± 0.01	0.49 ± 0.08$^{\$, \#}$
Glutathione disulphide	0.10 ± 0.01	1.58 ± 0.05$^{\$}$	4.51 ± 0.74$^{\#}$

[$] Significantly different compared to the Lizard

[£] Significantly different compared to Pigeon

[#] Significantly different compared to Toad

Table 5 Oxidative stress markers in the kidney of Lizard, *Slerophrys perreti* and *Columba unicinta*

Oxidative stress markers	Lizard	Toad	Pigeon
Malondialdehyde (mM)	1.58 ± 0.05	2.18 ± 0.04	6.62 ± 0.72$^{\$, \#}$
Catalase (u/mg)	1.82 ± 0.34	15.26 ± 1.38$^{\$, £}$	7.90 ± 0.59
Superoxide dismutase	0.17 ± 0.06	9.20 ± 2.24$^{\$}$	2.10 ± 0.10
Reduced glutathione	0.13 ± 0.01	0.16 ± 0.13	0.15 ± 0.01
Glutathione disulphide	0.07 ± 0.01	1.45 ± 0.11$^{\$,}$	1.33 ± 0.03$^{\$}$

$ Significantly different compared to the Lizard

£ Significantly different compared to Pigeon

\# Significantly different compared to Toad

Table 6 Haematology parameters in Lizard, Toad and Pigeon

Variables	Lizard	Toad	Pigeon
Packed cell volume (%)	40.60 ± 4.42	35.80 ± 1.17	51.00 ± 1.37$^{\$, \#}$
Red blood cell count	2.30 ± 0.19	5.28 ± 0.30$^{\$}$	5.95 ± 0.60$^{\$}$
Haemoglobin concentration	5.94 ± 0.43	10.00 ± 0.70	14.60 ± 0.74$^{\$, \#}$
Total white blood cell	1.60 ± 0.17	5.38 ± 0.44	12.87 ± 1.16
Glycated haemoglobin	3.74 ± 0.32	3.28 ± 0.28	3.68 ± 0.30

$ Significantly different compared to the Lizard

\# Significantly different compared to Toad

of Lizard and *Columba unicinta*. Moreover, the level of superoxide dismutase was higher in *Slerophrys perreti*'s kidney, compared with the levels observed in the kidneys of Lizard and *Columba unicinta*. There was no significant difference in the levels of reduced glutathione in the kidney across the groups. However, glutathione disulphide was significantly higher in *Slerophrys perreti* and *Columba unicinta*, compared with Lizard.

Comparative analyses of hematology parameters in Lizard, Toad and Pigeon

The results in Table 6 showed that there was no significant difference in the packed cell volume of Lizard and Toad; however, there was a significant difference in the packed cell volume of Toad when compared to that of Lizard and Pigeon.

Red blood cells in Toad and Pigeons were significantly higher, compared with those of Lizards.

Haemoglobin concentration was higher in Pigeons, compared with both Lizards and Toads. Also, haemoglobin concentration was higher in the Toad, compared with the Lizard. The total white blood cells in Pigeons were significantly higher, compared with Toads and Lizards. There was no significant difference in glycated haemoglobin across the groups.

Table 7 Differential white blood cell count in Lizard, Toad and Pigeon

Type of white blood cell	Lizard	Toad	Pigeon
Lymphocytes (%)	64.32 ± 1.03$^{£, \#}$	49.21 ± 1.01	46.68 ± 1.48
Neutrophils (%)	17.42 ± 0.35$^{£, \#}$	14.50 ± 0.58$^{\$, £}$	41.25 ± 1.76$^{\$, \#}$
Eosinophils (%)	10.06 ± 0.37$^{£}$	10.85 ± 0.98$^{£}$	4.46 ± 0.53
Basophils (%)	7.38 ± 0.35$^{£}$	10.22 ± 0.63$^{\$, £}$	0.68 ± 0.23
Monocytes (%)	0.58 ± 0.06	15.20 ± 0.55$^{\$, £}$	6.90 ± 0.55$^{\$}$

$ Significantly different compared to the Lizard

£ Significantly different compared to Pigeon

\# Significantly different compared to Toad

Table 8 Lipid Profile in Lizard, Toad and Pigeon

Variables	Lizard	Toad	Pigeon
Total cholesterol (mg/dl)	269.00 ± 43.10 $^{£, \#}$	200.60 ± 28.50	127.60 ± 29.69
Triglyceride (mg/dl)	162.70 ± 22.77	165.80 ± 11.71	237.22 ± 21.25$^{\$, \#}$

$ Significantly different compared to the Lizard

£ Significantly different compared to Pigeon

\# Significantly different compared to Toad

Comparative analyses of differential white blood cell count in Lizard, Toad and Pigeon

The lymphocyte count was significantly higher in the Lizard compared with that of the Toad and Pigeon. The neutrophils count was significantly higher in Pigeon compared with the neutrophils in Toads and Lizards. Also, neutrophil count was significantly higher in Lizards compared with Toads. Eosinophils count was significantly higher in Lizards and Toads compared with that of the Pigeon.

There was a significant increase in the basophile count of Lizards and Toad compared with that of Pigeons. However, there was no significant difference in the basophile count between Lizards and Toads. Monocytes in Toad and Pigeons are significantly higher when compared with that of Lizards. Also, monocytes in the Toad were significantly higher compared with that of Pigeons. The differential white blood cell count results are displayed in Table 7.

Comparative analyses of lipid profile in Lizard, Toad and Pigeon

There was a significant increase in the total cholesterol of Lizards, compared with that of Toad and Pigeons. However, there was no significant difference in the triglycerides of the Lizard, Toad, and Pigeon (Table 8).

Discussion

This study aims to investigate the adaptive strategies of three species: Toad, Lizard and Pigeon—to their environments by comparing their physiological responses, specifically: renal function and electrolyte balance, oxidative stress markers and antioxidant defences, liver enzyme activities and haematological parameters responses.

The body temperature in Pigeon was significantly higher compared with what was observed in Toad and Lizard. This agrees with the previous findings (Seebacher, 2005; Calder & Schmidt-Nielsen, 1967). Studies have shown that the aquatic environment contributes to the temperature regulation in reptiles and amphibian animals (Hochscheid et al., 2004; Manning & Grigg, 1997). The reason for increased body temperature in Pigeon is the higher rate of metabolism and lower rate of heat loss in birds (Mcnab, 1966). The respiratory rate in Lizard, Toad and Pigeon is not different. However, the respiratory rate of Pigeon observed in this study is higher than what was previously reported by Calder and Schmidt-Nielsen. Creatinine may be an index for the quantity of muscle mass and is also important in assessing renal function. Creatinine is important in hibernating; hibernators undergo torpor followed by arousal (Jani et al., 2012). During torpor, hibernators undergo profound physiological changes, reducing their heart rate (Zatzman, 1984), respiratory rate (Deavers and Musacchia, 1980) and their metabolic rate (Geiser, 2004), while during arousal; organs undergo rapid metabolic reactivation, reperfusion, and rewarming to near normal levels (Carey, 2003). The increased creatinine levels in Lizard suggest that Lizard is great hibernators which serve as an adaptive mechanism for their survival. This is contrary to the previous studies which showed that Toads have similar levels of creatinine with the amphibians but are lower compared to that of birds (Coppo et al., 2005). In terms of adaptation, it means that the Lizards have higher muscle mass, renal adaptation and hibernating mechanism, compared to Toad *and* Pigeon in the rainy season of tropical savannah climate.

The liver produces urea by the breakdown of amino acids. There is an increased urea production when endogenous catabolism is increased and after a protein-rich meal. The end-product of ammonia metabolism in animals is urea usually excreted in urine. Conversion of ammonia to urea is a special mechanism done by animals to eliminate ammonia from their system to achieve high osmolality of their body fluids (Molnar & Gair, 2015).

The high serum urea level in Pigeons was in agreement with the studies which showed that Fowls and Pigeons have significantly higher serum urea compared with Toads (Coppo et al., 2005). It has been established that in birds the urea cycle, which breaks down ammonia to produce urea, is incomplete (Griminger & Scanes, 1986).

The main source of fuel for cells in the body is glucose and its normal homeostasis is essential for maintaining health. Glucose is important in energy production through cellular oxidation (Braun & Sweazea, 2008). The higher level of blood glucose in Pigeons agrees with the findings which showed that Pigeons which are non-aquatic animals have significantly higher levels of blood glucose compared with frogs (Coppo et al., 2005). It has been shown that amphibians have low blood glucose levels during the stage previous to winter lethargy which is an adaptive mechanism for glycogen storage in the liver (Rocha & Branco, 1998). Studies have it that birds are generally insensitive to the regulation of glucose concentration by insulin (Braun & Sweazea, 2008). It is believed that Pigeons do not hibernate as Toads and Lizards and hence are more active than the two species. The significantly higher level of blood glucose in Pigeon is an adaptive mechanism for survival.

Calcium, sodium, magnesium and potassium are important electrolytes known for there are physiological roles in biologic and metabolic processes (Irnius et al., 2007). They help maintain a constant blood pH, regulating the osmotic gradients (Lee et al., 2013; Zamboni et al., 2012 and Zeneli & Daci, 2014). Higher levels of electrolytes were seen in Toad, suggesting that Toad has more proper hydration status and/or adaptive mechanism during the rainy season in tropical savannah climate.

The hypernatremia in Toad and Pigeon was also supported by previous study (Coppo, 2001). This may be due to high internal and low external osmolarity which initiates overhydration (entry of water by osmotic gradient) and electrolyte loss (diffusion by concentration gradient) and homeostasis is regulated by sufficient hypotonic urine, high electrolytes tubular absorption and salt cutaneous absorption (Randall et al., 2022). The insignificant difference in blood potassium levels across the group in this study was in agreement with a previous study (Coppo, 2001). In this study, Toad has significantly higher levels of chloride compared with Lizard and Pigeon. However, frogs have been reported to have higher levels of chloride compared with Toads (Coppo, 2001). Toad has significantly higher levels of magnesium compared with *agama* and Pigeon. However, this is contrary to a previous study which showed that there is no significant difference between Toads and birds (Coppo, 2001).

ALP is important for breaking down proteins. A higher level of ALP was seen in Pigeon compared with Toad and Lizards. This result agrees with the previous studies which showed that birds have significantly higher levels of serum alkaline phosphatase when compared with frogs

(Coppo, 2001). Studies have shown that birds have high serum ALP because of an increase in liver metabolism (Szabó et al., 2005). The observed results in ALT level, AST level, total protein, level of albumin and level of GGT were not significant.

There is a vital balance between the body's antioxidant defence and the generation of reactive oxygen species (ROS). Oxidative stress occurs in the event of the failure of the body to detoxify the RO and/or the antioxidant capacity of the cell is less compared to the generation of ROS. The first line of cellular defence mechanisms is the antioxidant which acts against oxidative stress (Winston & Di Giulio, 1991). Antioxidants act by scavenging ROS and reactive nitrogen species associated with oxidative stress experienced by organisms living in an aerobic environment.

Antioxidants function by eliminating reactive oxygen species (ROS) and reactive nitrogen species linked to oxidative stress encountered by organisms living in an oxygen-rich environment. MDA is a widely accepted representative marker for oxidative stress. It can trigger Kupffer cells to release various cytokines and manage the differentiation, proliferation and collagen synthesis of hepatic stellate cells (HSC) which helps the body mop up the excess ROS (Onadeko et al., 2014; Stacy et al., 2011). MDA results from lipid peroxidation of polyunsaturated fatty acid (Fenoglio et al., 2006). Increased malondialdehyde (MDA) in the Toad liver and Pigeon kidney may be an indication of reduced perfusion or oxygen delivery to them.

Superoxide dismutase (SOD) stands as the initial detoxifying enzyme and the most potent cell antioxidant. SOD is an essential endogenous antioxidant enzyme that serves as a constituent of the primary defence system against reactive oxygen species (ROS). Its role involves catalysing the dismutation of two molecules of superoxide anion (*O2) into hydrogen peroxide (H_2O_2) and molecular oxygen (O2), effectively making the potentially harmful superoxide anion less dangerous. SOD plays a critical role as the primary driving force in cellular or bodily adaptation to various stress conditions. Notably, during stressful situations, there is an adaptive mechanism of additional SOD synthesis to reduce ROS generation.

Catalase is a prevalent enzyme present in almost all living organisms exposed to oxygen which is actively involved in catalysing the breakdown of hydrogen peroxide into water and oxygen. This enzyme plays a crucial role in safeguarding the cell against oxidative damage occurring on exposure to ROS. From the result, catalase in the liver and kidney of Pigeon and Lizard, respectively, was significantly lower when compared to that of Toad. The decrease in the level of catalase is an indication of oxidative stress, thus reducing the oxidative stress effects on the liver and kidney.

Glutathione disulphide (GSSG) originates from the combination of two glutathione molecules (Davey et al., 2005). Within the living cell, glutathione disulphide transforms into two glutathione molecules through the donation of reducing equivalents from the coenzyme NADPH. The enzyme responsible for this reaction is glutathione reductase (Meister & Anderson, 1983). Antioxidant enzymes, like glutathione peroxidases and peroxiredoxins, produce glutathione disulphide while reducing peroxides, such as hydrogen peroxide (H2O2) and organic hydroperoxides (ROOH).

(Deneke & Fanburg, 1989). The increase in glutathione and glutathione disulphide in Pigeons is an indication of reduced oxidative stress in the liver of the Pigeon, while reduction in Pigeon kidney is an indication of increased oxidative stress. This follows the same trend in the Lizard and Toad.

Enzymes can be divided into extracellular or intracellular, where the first line of defence against ROS is on the surface of a cell such as SOD, representing a protective mechanism within cells. However, in aquatic animals, the water-soluble antioxidant glutathione (GSH) is present in micromolar amounts (Dickinson & Forman, 2002).

It is important to establish a reference range of the haematological parameters of the Lizard, Toad and Pigeon in the tropical rain forest in the diagnostic and prevention of diseases, in health monitoring and the detection of the ecological and geographical differences among various species. During migration, birds are exposed to different stress situations such as high metabolic demands, physical activity, food quality and quantity environmental contaminants which to induce changes in blood parameters that may lead to migratory and breeding constraints (Colin & Peter, 2005). The increased PCV level of Pigeon could be attributed to the stress situation encountered by bird during migration. The stress of moving at high altitudes with low oxygen could lead to hypoxia that causes an increase in their RBC production and subsequent increase in the PCV. This automatically leads to an increase in haemoglobin concentration, WBC as observed in this study. Lymphocytes were the dominant cell among the various types of leucocytes. Elevated leucocyte number (leucocytosis) can be symptomatic of stress syndrome and inflammatory/infective and neoplastic processes (Ots et al., 1998).

Conclusions

From this study, the elevated level of lymphocytes in Lizard and eosinophil, basophile and monocytes in Toad suggests that Lizards and Toad are more vulnerable to inflammation. The high value of cholesterol in Lizard and triglyceride in Pigeon as observed in this study may be related to the degree of stress. Also, the activation of antioxidant systems under comparative study is a part of the survival strategy of the animals like amphibians, reptiles and aves to adapt to various environmental problems.

Abbreviations

ANOVA	Analysis of variance
AST	Aspartate aminotransferase
CAT	Catalase
GGT	Gamma-glutamyl transferase
GSH	Glutathione
*O2	Superoxide anion
ALP	*Alkaline phosphatase*
ALT	Alanine transaminase
Cl^-	Chloride ions
EDTA	Ethylenediaminetetraacetic acid
GSSG	Glutathione disulfide
H_2O_2	Hydrogen peroxide
HSC	Hepatic stellate cells
k^+	Potassium
MDA	Malondialdehyde
Mg^+	Magnesium ion
Na^+	Sodium ion
NADPH	Nicotinamide adenine dinucleotide phosphate
PCV	Packed cell volume
RH	Relative humidity
ROOH	Hydroperoxides
ROS	*Reactive oxygen species*
SOD	*Superoxide dismutase*
TP	Total protein
WBC	White blood cells

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